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(57) Abstract

The present invention pertains to polynucleotides derived from *staphylococcal* genes encoding resistance to streptogramin A or to streptogramin B and chemically related compounds. This invention also relates to the use of the polynucleotides as oligonucleotide primers or probes for detecting *Staphylococcal* strains that are resistant to streptogramin A or to streptogramin B and related compounds in a biological sample. In another embodiment, the present invention is directed to the full length coding sequences of the *staphylococcal* genes encoding for resistance to streptogramin A or to streptogramin B from *Staphylococcus* and to the polypeptides expressed by these full length coding sequences. Further, this invention relates to the use of the expressed polypeptides to produce specific monoclonal or polyclonal antibodies that serve as detection means in order to characterize any *staphylococcal* strain carrying genes encoding resistance to streptogramin A or to streptogramin B.

# POLYNUCLEOTIDES AND THEIR USE FOR DETECTING RESISTANCE TO STREPTOGRAMIN A OR TO STREPTOGRAMIN B AND RELATED COMPOUNDS

The present invention pertains to polynucleotides derived from *staphylococcal* genes encoding resistance to streptogramin A or to streptogramin B and chemically related compounds. This invention also relates to the use of the polynucleotides as oligonucleotide primers or probes for detecting *Staphylococcal* strains that are resistant to streptogramin A or to streptogramin B and related compounds in a biological sample.

In another embodiment, the present invention is directed to the full length coding sequences of the *staphylococcal* genes encoding for resistance to streptogramin A or to streptogramin B from *Staphylococcus* and to the polypeptides expressed by these full length coding sequences.

Further, this invention relates to the use of the expressed polypeptides to produce specific monoclonal or polyclonal antibodies that serve as detection means in order to characterize any *staphylococcal* strain carrying genes encoding resistance to streptogramin A or to streptogramin B.

The present invention is also directed to diagnostic methods for detecting specific strains of *Staphylococcus* expected to be contained in a biological sample. The diagnostic methods use the oligonucleotide probes and primers as well as the antibodies of the invention.

Streptogramins and related compounds (antibiotics) produced by streptomycetes can be classified as A and B compounds according to their basic primary structures (Cocito, 1979). Compounds of the A group, including streptogramin A (SgA), pristinamycin IIA (PIIA), virginiamycin M, mikamycin A, or synergistin A, are polyunsaturated cyclic macrolactones. Compounds of the B group, including streptogramin B (SgB), pristinamycin B (PIB), virginiamycin S, mikamycin B, and synergistin B, are cyclic peptidic macrolactones (Cocito, 1979). Compounds of both groups, A and B, bind different targets in the peptidyltransferase domain of the 50S

ribosomal subunit and inhibit protein elongation at different steps (Aumercier et al., 1992; Di Giambattista et al., 1989).

A decrease in the dissociation constant of PIB is observed in the presence of PIIA because this latter antibiotic provokes a conformational modification of the bacterial ribosome at the binding sites of these molecules. Thus, A and B compounds, which are bacteriostatic when used separately, act synergistically when combined and become bactericidal, mainly against Gram-positive bacteria.

Natural mixtures such as pristinamycin (Pt), synergistin, virginiamycin and mikamycin, are used orally and topically. A semi-synthetic injectable streptogramin, RP59500, consisting of a mixture of derivatives of A and B compounds (Dalfopristin and Quinupristin, respectively) is currently undergoing *in vivo* experimental and clinical trials (J. Antimicrob. Agents Chemother. 30 (Suppl. A), entire volume, 1992; Entenza et al., 1995; Fantin et al., 1995; Griswold et al., 1996; Torralba et al., 1995). Staphylococcal resistance to synergistic mixtures of A and B compounds (Pt MIC $\geq$ 2 $\mu$g/ml) is always associated with resistance to A compounds (PIIA MIC $\geq$ 8 $\mu$g/ml), but not necessarily with resistance to B compounds (Allignet et al., 1996).

To date, four genes encoding resistance to A compounds have been isolated from staphylococcal and enterococcal plasmids. The genes *vat* (Allignet et al., 1993), *vat*B (Allignet and El Solh, 1995), and *sat*A (Rende-Fournier et al., 1993) encode related acetyltransferases (50.4-58.3 % amino acids), which inactivate streptogramin A and similar compounds. The staphylococcal gene *vga* (Allignet et al., 1992) encodes an ATP-binding protein probably involved in the active efflux of A compounds. Nevertheless, there continues to exist a need in the art for polynucleotides specific for *Staphylococcus* resistant to streptogramin A and/or B and related compounds.

## SUMMARY OF THE INVENTION

Accordingly, this invention aids in fulfilling this need in the art. In particular, this invention provides a purified peptide comprising an amino acid sequence selected from the group consisting of :

a) SEQ ID NO: 4 which corresponds to the complete amino acid sequence of Vga B or fragments derived from SEQ ID NO: 4 containing at least 10 amino acids;

b) SEQ ID NO: 5 which corresponds to the complete amino acid sequence of Vat C or fragments derived from SEQ ID NO: 5 containing at least 10 amino acids;

c) SEQ ID NO: 6 which corresponds to the complete amino acid sequence of Vgb B or fragments derived from SEQ ID NO: 6 containing at least 10 amino acids;

d) SEQ ID NO: 7 which corresponds to the complete amino acid sequence of Vgb B;

e) SEQ ID NO: 8 which corresponds to a fragment of the amino acid sequence of Vga B;

f) SEQ ID NO: 9 which corresponds to a fragment of the amino acid sequence of Vat C; and

g) SEQ ID NO: 10 which corresponds to a fragment of the amino acid sequence of Vat C.

This invention additionally provides a purified polynucleotide comprising the nucleotide sequence selected from the group consisting of :

a) SEQ ID NO: 1 which corresponds to the complete nucleic acid sequence of vga B or fragments derived from SEQ ID NO: 1 containing 15 to 40 nucleotides;

b) SEQ ID NO: 2 which corresponds to the complete nucleic acid sequence of vat C or fragments derived from SEQ ID NO: 2 containing 15 to 40 nucleotides;

c) SEQ ID NO: 3 which corresponds to the complete nucleic acid sequence of vgb B or fragments derived from SEQ ID NO: 3 containing 15 to 40 nucleotides;

d) SEQ ID NO: 11 which corresponds to the nucleic acid sequence encoding the polypeptide of SEQ ID NO: 7;

e) SEQ ID NO: 12 which corresponds to the nucleic acid sequence encoding the polypeptide of SEQ ID NO: 8 ;

f) SEQ ID NO: 13 which corresponds to the nucleic acid sequence encoding the polypeptide of SEQ ID NO: 9; and

g) SEQ ID NO: 14 which corresponds to the nucleic acid sequence encoding the polypeptide of SEQ ID NO: 10.

Furthermore, this invention includes a purified peptide comprising the amino acid sequence encoded by the nucleotide sequence selected from the group consisting of :

a) SEQ ID NO: 1,

b) SEQ ID NO: 2,

c) SEQ ID NO: 3,

d) SEQ ID NO: 11,

e) SEQ ID NO: 12,

f) SEQ ID NO: 13, and

g) SEQ ID NO: 14.

This invention also provides a composition comprising purified polynucleotide sequences including at least one nucleotide sequence selected from the group consisting of polynucleotides, genes or cDNA of *vgaB*, *vatC*, and *vgbB*, which are useful for the detection of resistance to streptogramin A and/or to streptogramin B and related compounds. This invention further provides a composition comprising purified amino acid sequences including at least an amino acid sequence from a polypeptide encoded by a polynucleotide selected from the group consisting of polynucleotides, genes or cDNA of *vgaB*, *vatC*, and *vgbB*, which are useful for the detection of resistance to streptogramin A and/or to streptogramin B and related compounds.

In another embodiment, this invention provides a composition of polynucleotide sequences encoding resistance to streptogramins and related compounds, or inducing this resistance in Gram-positive bacteria, wherein the composition comprises a combination of at least two of the following nucleotide sequences: a) a nucleotide sequence encoding an acetyltransferase conferring resistance to streptogramin A and related compounds, b) a nucleotide sequence encoding a molecule containing ATP binding motifs conferring resistance to streptogramin A and related compounds; and c) a nucleotide sequence encoding a lactonase conferring resistance to streptogramin B and related compounds.

Furthermore, this invention provides a composition of polynucleotide sequences, wherein the sequence encoding a molecule containing ATP binding motifs confers resistance to *Staphylococci* and particularly to *S. aureus*, and wherein the polynucleotide sequence corresponds to a *vgaB* nucleotide sequence represented by SEQ ID NO: 1 or a sequence having at least 70% homology with *vgaB* complete nucleotide sequence, or to a polynucleotide hybridizing with SEQ ID NO: 1 under stringent conditions, or to a fragment containing between 20 and 30 nucleotides of SEQ ID NO: 11 or SEQ ID NO: 12, or wherein the polynucleotide sequence encodes a polypeptide having at least 60% homology with the complete SEQ ID NO: 4 or with SEQ ID NO: 7 or SEQ ID NO: 8.

Furthermore this invention relates to a composition of polynucleotide sequences, wherein the sequence encoding an acetyltransferase confers resistance to streptogramin A and related compounds in *Staphylococci*, and particularly in *S. cohnii*, and wherein the polynucleotide sequence corresponds to a *vatC* nucleotide sequence represented by SEQ ID NO: 2 or a sequence having at least 70% homology with *vatC* complete nucleotide sequence, or to a polynucleotide hybridizing with SEQ ID NO: 2 under stringent conditions, or to a fragment containing between 20 and 30 nucleotides of SEQ ID NO: 13 or SEQ ID NO: 14, or wherein the polynucleotide sequence encodes a polypeptide having at least 60% homology with the complete SEQ ID NO: 5 or with SEQ ID NO: 9 or SEQ ID NO: 10.

This invention also provides a composition of polynucleotide sequences, wherein the sequence encoding a lactonase confers resistance to streptogramin B and related compounds in *Staphylococci* and particularly in *S. cohnii*, and wherein the polynucleotide sequence corresponds to a *vgbB* nucleotide sequence represented in SEQ ID NO: 3 or a sequence having at least 70% homology with *vgbB* complete nucleotide sequence, or to a polynucleotide hybridizing with SEQ ID NO: 3 under stringent conditions, or to a fragment containing between 20 and 40 nucleotides of SEQ ID NO: 3, or wherein the polynucleotide sequence encodes a polypeptide having at least 60% homology with the complete SEQ ID NO: 6.

The invention also contemplates a composition of polynucleotide sequences, wherein at least a *vatB* nucleotide sequence encoding an acetyltransferase conferring resistance to streptogramin A and related compounds is included in addition to a *vgaB* nucleotide sequence encoding a molecule containing ATP binding motifs conferring resistance to streptogramin A.

Additionally, the invention includes a purified polynucleotide that hybridizes specifically under stringent conditions with a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, and SEQ ID NO: 14.

The invention further includes polynucleotide fragments comprising at least 10 nucleotides capable of hybridization under stringent conditions with any one of the nucleotide sequences enumerated above.

In another embodiment of the invention, a recombinant DNA sequence comprising at least one nucleotide sequence enumerated above and under the control of regulatory elements that regulate the expression of resistance to antibiotics of the streptogramin family in a defined host is provided.

Furthermore, the invention includes a recombinant vector comprising the recombinant DNA sequence noted above, wherein the vector comprises the plasmid pIP1633 or plasmid pIP1714.

The invention also includes a recombinant cell host comprising a polynucleotide sequence enumerated above or the recombinant vector defined above.

In still a further embodiment of the invention, a method of detecting bacterial strains that contain the polynucleotide sequences set forth above is provided.

Additionally, the invention includes kits for the detection of the presence of bacterial strains that contain the polynucleotide sequences set forth above.

The invention also contemplates antibodies recognizing peptide fragments or polypeptides encoded by the polynucleotide sequences enumerated above.

Still further, the invention provides for a screening method for active antibiotics and/or molecules for the treatment of infections due to Gram-positive bacteria, particularly staphylococci, based on the detection of activity of these antibiotics and/or molecules on bacteria having the resistance phenotype to streptogramins.

It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

## BRIEF DESCRIPTION OF THE DRAWINGS

This invention will be more fully described with reference to the drawings in which:

FIG. 1A and 1B are the restriction maps of the 5.5 kb *Bgl*II fragment and of the 2.4 kb *Hin*dIII-*Hae*III fragment of pIP1633, respectively. Both fragments confer resistance to streptogramin A and related compounds. The strategy for sequencing the 2.4 kb *HindIII-Hae*III fragment is given in Fig. 1B. Restriction enzyme abbreviations: Ba, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; X, *Xba*I.

FIG. 2 is the nucleotide sequence and deduced amino acid sequence of 2411 nucleotides from pIP1633, which contains the gene *vgaB* of *S. aureus* conferring resistance to streptogramin A and related compounds. The putative ribosome binding site (RBS) is underlined. The amino acids are aligned with the second nucleotide of each

codon. Asterisks indicate the in-frame stop codons. The A and B ATP-binding motifs described by Walker et al. (1982) and detected within each of the two ATP-domains are boxed. The conserved motif SGG of the two copies of loop 3 described by Hyde et al. (1990) is underlined. Relevant restriction sites are shown.

FIG. 3 is the amino acid sequence alignment of the predicted 60 and 61 kDa proteins encoded by Vga (Allignet et al., 1992, accession No: m90056) and VgaB (FIG.2), respectively. Identical residues are indicated by asterisks and conservative changes are shown by single dots. The A and B motifs of Walker et al. (1982) are in bold type (WA, WB). The conserved motif SGG of the two copies of loop 3 described by Hyde et al. (1990) is underlined.

FIG. 4 is a restriction map of the plasmid pIP1714 carrying the genes *vatC* and *vgbB* as well as the genes *pre* and *repB* of *S. cohnii* strain BM10711 resistant to the synergistic mixtures of streptogramins A and B.

FIG. 5 is the nucleotide sequence and deduced amino acid sequence of 1727 nucleotide from pIP1714, which contains the gene *vgbB* and *vatC* of *S. cohnii*. Relevant restriction sites are shown.

FIG. 6 A, 6B, and 6C represent oligonucleotide primers for hybridization under stringent conditions with *vatC*, *vgbB*, and *vgaB* respectively.

FIG. 7 represents SEQ ID NOs: 1-14.

## DETAILED DESCRIPTION OF THE INVENTION

It has now been determined that bacteria from the *Staphylococcus* genus carry a *vga*B gene, which encodes a putative ATP-binding protein that confers resistance to streptogramin A and structurally similar compounds. It has also now been determined that bacteria from the *Staphylococcus* genus carry a *vgb*B gene, which encodes a lactonase that confers resistance to streptogramin B and structurally similar compounds, and a *vat*C gene, which encodes an acetyltransferase that confers resistance to streptogramin A and structurally similar compounds.

Novel polynucleotides corresponding to the *vga*B, *vgb*B, and *vat*C genes from various strains of *Staphylococcus* have been isolated and sequenced, and it has been surprisingly demonstrated that these new polynucleotides make it possible to design oligonucleotide probes or primers. These polynucleotides include the following:

a) SEQ ID NO: 1,

b) SEQ ID NO: 2,

c) SEQ ID NO: 3,

d) SEQ ID NO: 11,

e) SEQ ID NO: 12,

f) SEQ ID NO: 13, and

g) SEQ ID NO: 14.

This invention provides specific pairs of oligonucleotide primers or probes that hybridize specifically, under stringent hybridization conditions as defined hereinafter, to the nucleic acid (RNA or DNA) from a particular strain of the *Staphylococcus* genus. These oligonucleotide primers include the following:

a) Oligo I 5'-AAGTCGACTGACAATATGAGTGGTGG-3'
Oligo II 5'-CTGCAGATGCCTCAACAGCATCGATATCC-3'

b) Oligo III 5'- ATGAATTCGCAAATCAGCAAGG -3'
Oligo IV 5'- TCGTCTCGAGCTCTAGGTCC -3'

c) Oligo V 5'- CAGCAGTCTAGATCAGAGTGG -3'
Oligo VI 5'- CATACGGATCCACCTTTTCC -3'.

In a specific embodiment of the present invention, the purified polynucleotides useful for detecting *Staphylococcal* strains can be used in combination in order to detect bacteria belonging to *Staphylococci* in a biological sample. Thus, the present invention also provides detection methods and kits comprising combinations of the purified polynucleotides according to the invention. The purified oligonucleotides of the invention are also useful as primers for use in amplification reactions or as nucleic acid probes.

By "polynucleotides" according to the invention is meant the sequences referred to as SEQ ID NOs: 1, 2, 3, OR 11, 12, 13, 14 and the complementary sequences and/or the sequences of polynucleotides which hybridize to the referred sequences in high stringent conditions and which are used for detecting *staphylococcal* strains carrying a gene encoding resistance to streptogramin A or to streptogramin B.

By "active molecule" according to the invention is meant a molecule capable of inhibiting the activity of the purified polypeptide as defined in the present invention or capable of inhibiting the bacterial culture of *staphylococcal* strains.

Thus, the polynucleotides of SEQ ID NOs: 1-3 and 11-14 and their fragments can be used to select nucleotide primers notably for an amplification reaction, such as the amplification reactions further described.

PCR is described in the U.S. Patent No. 4,683,202 granted to Cetus Corp. The amplified fragments may be identified by agarose or polyacrylamide gel electrophoresis, or by a capillary electrophoresis, or alternatively by a chromatography technique (gel filtration, hydrophobic chromatography, or ion exchange chromatography). The specificity of the amplification can be ensured by a molecular hybridization using as nucleic probes the polynucleotides of SEQ ID NOs: 1-3 and 11-14 and their fragments, oligonucleotides that are complementary to these polynucleotides or fragments thereof, or their amplification products themselves.

Amplified nucleotide fragments are useful as probes in hybridization reactions in order to detect the presence of one polynucleotide according to the present invention or in order to detect the presence of a bacteria of *Staphylococcal* strain carrying genes encoding resistance to streptogramin A or streptogramin B, in a biological sample. This invention also provides the amplified nucleic acid fragments ("amplicons") defined herein above. These probes and amplicons can be radioactively or non-radioactively labeled, using for example enzymes or fluorescent compounds.

Preferred nucleic acid fragments that can serve as primers according to the present invention are the following:

polynucleotides of sequence SEQ ID NOs: 1-3 and 11-14; and

polynucleotides having a length from 20 to 30 consecutive nucleotides from a polynucleotide selected from the group consisting of polynucleotides of sequences SEQ ID NO: 11 to SEQ ID NO: 14 or from 20 to 40 consecutive nucleotides from a polynucleotide of SEQ ID NO: 3

The primers can also be used as oligonucleotide probes to specifically detect a polynucleotide according to the invention.

Other techniques related to nucleic acid amplification can also be used and are generally preferred to the PCR technique. The Strand Displacement Amplification (SDA) technique (Walker et al., 1992) is an isothermal amplification technique based on the ability of a restriction enzyme to cleave one of the strands at a recognition site (which is under a hemiphosphorothioate form), and on the property of a DNA polymerase to initiate the synthesis of a new strand from the 3' OH end generated by the restriction enzyme and on the property of this DNA polymerase to displace the previously synthesized strand being localized downstream.

The SDA amplification technique is more easily performed than PCR (a single thermostated water bath device is necessary), and is faster than the other amplification methods. Thus, the present invention also comprises using the nucleic acid fragments according to the invention (primers) in a method of DNA or RNA amplification according to the SDA technique. The polynucleotides of SEQ ID NOs: 1-3 and 11-14 and their fragments, especially the primers according to the invention, are useful as technical means for performing different target nucleic acid amplification methods such as:

- TAS (Transcription-based Amplification System), described by Kwoh et al. in 1989;

- SR (Self-Sustained Sequence Replication), described by Guatelli et al. in 1990;

- NASBA (Nucleic acid Sequence Based Amplification), described by Kievitis et al. in 1991; and

- TMA (Transcription Mediated Amplification).

The polynucleotides of SEQ ID NOs: 1-3 and 11-14 and their fragments, especially the primers according to the invention, are also useful as technical means for performing methods for amplification or modification of a nucleic acid used as a probe, such as:

- LCR (Ligase Chain Reaction), described by Landegren et al. in 1988 and improved by Barany et al. in 1991, who employ a thermostable ligase;

- RCR (Repair Chain Reaction), described by Segev et al. in 1992;

- CPR (Cycling Probe Reaction), described by Duck et al. in 1990; and

- Q-beta replicase reaction, described by Miele et al. in 1983 and improved by Chu et al. in 1986, Lizardi et al. in 1988, and by Burg et al. and Stone et al. in 1996.

When the target polynucleotide to be detected is RNA, for example mRNA, a reverse transcriptase enzyme can be used before the amplification reaction in order to obtain a cDNA from the RNA contained in the biological sample. The generated cDNA can be subsequently used as the nucleic acid target for the primers or the probes used in an amplification process or a detection process according to the present invention.

Nucleic probes according to the present invention are specific to detect a polynucleotide of the invention. By "specific probes" according to the invention is meant any oligonucleotide that hybridizes with one polynucleotide of SEQ ID NOs: 1-3 and 11-14 and which does not hybridize with unrelated sequences. Preferred oligonucleotide probes according to the invention are oligonucleotides I-VI.

In a specific embodiment, the purified polynucleotides according to the present invention encompass polynucleotides having at least 80% homology in their nucleic acid sequences with polynucleotides of SEQ ID NO: 11 to SEQ ID NO: 14, at least 70% identity with SEQ ID NO: 1 to 3. By percentage of nucleotide homology according to the present invention is intended a percentage of identity between the corresponding bases of two homologous polynucleotides, this percentage of identity being purely statistical

and the differences between two homologous polynucleotides being located at random and on the whole length of said polynucleotides.

The oligonucleotide probes according to the present invention hybridize specifically with a DNA or RNA molecule comprising all or part of one polynucleotide among SEQ ID NOs: 1-3 and 11-14 under stringent conditions. As an illustrative embodiment, the stringent hybridization conditions used in order to specifically detect a polynucleotide according to the present invention are advantageously the following:

Prehybridization and hybridization are performed at 68°C in a mixture containing:

- 5X SSPE (1X SSPE is .3 M NaCl, 30 mM tri-sodium citrate
- 5X Denhardt's solution
- 0.5% (w/v) sodium dodecyl sulfate (SDS); and
- 100 $\mu g\ ml^{-1}$ salmon sperm DNA

The washings are performed as follows:

- Two washings at laboratory temperature for 10 min. in the presence of 2 x SSPE and 0.1 % SDS;
- One washing at 68°C for 15 min. in the presence of 1 x SSPE, .1% SDS; and
- One washing at 68°C for 15 min. in the presence of 0.1 x SSPE and 0.1 % SDS.

The non-labeled polynucleotides or oligonucleotides of the invention can be directly used as probes. Nevertheless, the polynucleotides or oligonucleotides are generally labeled with a radioactive element ($^{32}P$, $^{35}S$, $^{3}H$, $^{125}I$) or by a non-isotopic molecule (for example, biotin, acetylaminofluorene, digoxigenin, 5-bromodesoxyuridin, fluorescein) in order to generate probes that are useful for numerous applications. Examples of non-radioactive labeling of nucleic acid fragments are described in the French Patent No. FR 78 10975 or by Urdea et al. or Sanchez-Pescador et al. 1988.

Other labeling techniques can also be used, such as those described in the French patents 2 422 956 and 2 518 755. The hybridization step may be performed in different ways (Matthews et al. 1988). A general method comprises immobilizing the nucleic acid that has been extracted from the biological sample on a substrate (nitrocellulose, nylon, polystyrene) and then incubating, in defined conditions, the target nucleic acid with the probe. Subsequent to the hybridization step, the excess amount of the specific probe is discarded, and the hybrid molecules formed are detected by an appropriate method (radioactivity, fluorescence, or enzyme activity measurement).

Advantageously, the probes according to the present invention can have structural characteristics such that they allow signal amplification, such structural characteristics being, for example, branched DNA probes as those described by Urdea et al. in 1991 or in the European Patent No. 0 225 807 (Chiron).

In another advantageous embodiment of the present invention, the probes described herein can be used as "capture probes", and are for this purpose immobilized on a substrate in order to capture the target nucleic acid contained in a biological sample. The captured target nucleic acid is subsequently detected with a second probe, which recognizes a sequence of the target nucleic acid that is different from the sequence recognized by the capture probe.

The oligonucleotide fragments useful as probes or primers according to the present invention can be prepared by cleavage of the polynucleotides of SEQ ID NOs: 1-3 and 11-14 by restriction enzymes, as described in Sambrook et al. in 1989. Another appropriate preparation process of the nucleic acids of the invention containing at most 200 nucleotides (or 200 bp if these molecules are double-stranded) comprises the following steps:

- synthesizing DNA using the automated method of beta-cyanethylphosphoramidite described in 1986;
- cloning the thus obtained nucleic acids in an appropriate vector;

and

- purifying the nucleic acid by hybridizing to an appropriate probe according to the present invention.

A chemical method for producing the nucleic acids according to the invention, which have a length of more than 200 nucleotides (or 200 bp if these molecules are double-stranded), comprises the following steps:

- Assembling the chemically synthesized oligonucleotides having different restriction sites at each end;
- cloning the thus obtained nucleic acids in an appropriate vector; and
- purifying the nucleic acid by hybridizing to an appropriate probe according to the present invention.

The oligonucleotide probes according to the present invention can also be used in a detection device comprising a matrix library of probes immobilized on a substrate, the sequence of each probe of a given length being localized in a shift of one or several bases, one from the other, each probe of the matrix library thus being complementary to a distinct sequence of the target nucleic acid. Optionally, the substrate of the matrix can be a material able to act as an electron donor, the detection of the matrix positions in which hybridization has occurred being subsequently determined by an electronic device. Such matrix libraries of probes and methods of specific detection of a target nucleic acid are described in the European patent application No. 0 713 016, or PCT Application No. WO 95 33846, or also PCT Application No. WO 95 11995 (Affymax Technologies), PCT Application No. WO 97 02357 (Affymetrix Inc.), and also in U.S. Patent No. 5,202,231 (Drmanac), said patents and patent applications being herein incorporated by reference.

The present invention also pertains to a family of recombinant plasmids containing at least a nucleic acid according to the invention. According to an advantageous embodiment, a recombinant plasmid comprises a polynucleotide of SEQ ID NOs: 1-3 and 11-14 or one nucleic fragment thereof. More specifically, the following plasmids are part of the invention: pIP1633 and pIP1714.

The present invention is also directed to the full length coding sequences of the *vgaB*, *vgbB*, and *vatC* genes from *Staphylococci* that are available using the purified polynucleotides according to the present invention, as well as to the polypeptide enzymes encoded by these full length coding sequences. In a specific embodiment of the present invention, the full length coding sequences of the *vgaB*, *vgbB*, and *vatC* genes are isolated from a plasmid or cosmid library of the genome of *Staphylococci* that have been screened with the oligonucleotide probes according to the present invention. The selected positive plasmid or cosmid clones hybridizing with the oligonucleotide probes of the invention are then sequenced in order to characterize the corresponding full length coding sequence, and the DNA insert of interest is then cloned in an expression vector in order to produce the corresponding ATP binding motif conferring resistance to streptogramin A and related compounds, acetyltransferase conferring resistance to streptogramin A and related compounds, or lactonase conferring resistance to streptogramin B and related compounds.

A suitable vector for the expression in bacteria and in particular in *E. coli*, is the pQE-30 vector (QIAexpress) that allows the production of a recombinant protein containing a 6xHis affinity tag. The 6xHis tag is placed at the C-terminus of the recombinant polypeptide ATP binding motif conferring resistance to streptogramin A and related compounds, acetyltransferase conferring resistance to streptogramin A and related compounds or lactonase conferring resistance to streptogramin B and related compounds, which allows a subsequent efficient purification of the recombinant polypeptide ATP binding motif conferring resistance to streptogramin A and related compounds, acetyltransferase conferring resistance to streptogramin A and related compounds, or lactonase conferring resistance to streptogramin B and related compounds by passage onto a nickel or copper affinity chromatography column. The nickel chromatography column can contain the Ni-NTA resin (Porath et al. 1975).

The polypeptides according to the invention can also be prepared by conventional methods of chemical synthesis, either in a homogenous solution or in solid phase. As an

illustrative embodiment of such chemical polypeptide synthesis techniques the homogenous solution technique described by Houbenweyl in 1974 may be cited.

The polypeptides according to the invention can be characterized by binding onto an immunoaffinity chromatography column on which polyclonal or monoclonal antibodies directed to a polypeptide among the ATP binding motif conferring resistance to streptogramin A and related compounds, acetyltransferase conferring resistance to streptogramin A and related compounds, or lactonase conferring resistance to streptogramin B and related compounds of the invention have previously been immobilized.

Another object of the present invention comprises a polypeptide produced by the genetic engineering techniques or a polypeptide synthesized chemically as above described.

The polypeptide ATP binding motif conferring resistance to streptogramin A and related compounds, acetyltransferase conferring resistance to streptogramin A and related compounds, or lactonase conferring resistance to streptogramin B and related compounds according to the present invention are useful for the preparation of polyclonal or monoclonal antibodies that recognize the polypeptides or fragments thereof. The monoclonal antibodies can be prepared from hybridomas according to the technique described by Kohler and Milstein in 1975. The polyclonal antibodies can be prepared by immunization of a mammal, especially a mouse or a rabbit, with a polypeptide according to the invention that is combined with an adjuvant, and then by purifying specific antibodies contained in the serum of the immunized animal on a affinity chromatography column on which has previously been immobilized the polypeptide that has been used as the antigen.

Consequently, the invention is also directed to a method for detecting specifically the presence of a polypeptide according to the invention in a biological sample. The method comprises:

a) bringing into contact the biological sample with an antibody according to the invention; and

b) detecting antigen-antibody complex formed.

Also part of the invention is a diagnostic kit for *in vitro* detecting the presence of a polypeptide according to the present invention in a biological sample. The kit comprises:

- a polyclonal or monoclonal antibody as described above, optionally labeled; and
- a reagent allowing the detection of the antigen-antibody complexes formed, wherein the reagent carries optionally a label, or being able to be recognized itself by a labeled reagent, more particularly in the case when the above-mentioned monoclonal or polyclonal antibody is not labeled by itself.

Indeed, the monoclonal or polyclonal antibodies according to the present invention are useful as detection means in order to identify or characterize a *Staphylococcal* strain carrying genes encoding resistance to streptogramin A or streptogramin B.

The invention also pertains to:

A purified polypeptide or a peptide fragment having at least 10 amino acids, which is recognized by antibodies directed against a polynucleotide sequence conferring resistance to streptogramin and related compounds, corresponding to a polynucleotide sequence according to the invention.

A polynucleotide comprising the full length coding sequence of a *Staphylococcus* streptogramin A and/or B resistant gene containing a polynucleotide sequence according to the invention.

A monoclonal or polyclonal antibody directed against a polypeptide or a peptide fragment encoded by the polynucleotide sequences according to the invention.

A method of detecting the presence of bacterium harboring the polynucleotide sequences according to the invention in a biological sample comprising:

a) contacting bacterial DNA of the biological sample with a primer or a probe according to the invention, which hybridizes with a nucleotide sequence encoding resistance to streptogramins;

b) amplifying the nucleotide sequence using said primer or said probe; and

c) detecting the hybridized complex formed between said primer or probe with the DNA.

A kit for detecting the presence of bacterium having resistance to streptogramin A and/or streptogramin B and harboring the polynucleotide sequences according to the invention in a biological sample, said kit comprising:

a) a polynucleotide probe according to the invention; and

b) reagents necessary to perform a nucleic acid hybridization reaction.

A kit for detecting the presence of bacterium having resistance to streptogramin A and harboring the polynucleotide sequences according to the invention in a biological sample, said kit comprising:

a) a polynucleotide probe according to the invention; and

b) reagents necessary to perform a nucleic acid hybridization reaction.

A method of screening active antibiotics for the treatment of the infections due to Gram-positive bacteria, comprising the steps of:

a) bringing into contact a Gram-positive bacteria having a resistance to streptogramin A or streptogramin B and related compounds and containing the polynucleotide sequences according to the invention with the antibiotic; and

b) measuring an activity of the antibiotic on the bacteria having a resistance to streptogramins and related compounds.

A method of screening for active synthetic molecules capable of penetrating into a bacteria of the family of staphylococci, wherein the inhibiting activity of these molecules is tested on at least a polypeptide encoded by the polynucleotide sequences according to the invention comprising the steps of:

a) contacting a sample of said active molecules with the bacteria;

b) testing the capacity of the active molecules to penetrate into the bacteria and the capacity of inhibiting a bacterial culture at various concentration of the molecules; and

c) choosing the active molecule that provides an inhibitory effect of at least 80% on the bacterial culture compared to an untreated culture.

An *in vitro* method of screening for active molecules capable of inhibiting a polypeptide encoded by the polynucleotide sequences according to the invention, wherein the inhibiting activity of these molecules is tested on at least said polypeptide, said method comprising the steps of:

a) extracting a purified polypeptide according to the invention;

b) contacting the active molecules with said purified polypeptide;

c) testing the capacity of the active molecules, at various concentrations, to inhibit the activity of the purified polypeptide; and

d) choosing the active molecule that provides an inhibitory effect of at least 80 % on the activity of the said purified polypeptide.

A composition of a polynucleotide sequence encoding resistance to streptogramins and related compounds, or inducing resistance in Gram-positive bacteria, wherein said composition comprises a nucleotide sequence corresponding to the resistance phenotype of the plasmid pIP1633 deposited with the C.N.C.M. under the Accession No. I-1768 and of the plasmid pIP1680 deposited with the C.N.C.M. under the Accession No. I-1767 and of the plasmid pIP1714 deposited with the C.N.C.M. under the number I-1877 on June 18, 1997.

A method of detecting the presence of bacterium harboring the polynucleotide sequences according to the invention in a biological sample, said method comprising the steps of:

a) contacting said sample with an antibody according to the invention that recognizes a polypeptide encoded by said polynucleotide sequences; and

b) detecting said complex.

A diagnostic kit for *in vitro* detecting the presence of bacterium harboring the polynucleotide sequences according to the invention in a biological sample, said kit comprising:

a) a predetermined quantity of monoclonal or polyclonal antibodies according to the invention;

b) reagents necessary to perform an immunological reaction between the antibodies and a polypeptide encoded by said polynucleotide sequences; and

c) reagents necessary for detecting said complex between the antibodies and the polypeptide encoded by said polynucleotide sequences.

The inhibiting activity of the molecules can be readily evaluated by one skilled in the art. For example, the inhibiting activity of Vga B can be tested by detecting its ATP hydrolysis as described in J.I. Ross et al. (1990), Mol. Microbiol. 4(7):1207-1214

regarding the rate evaluation of the active efflux of antibiotics from a cell. Ross et al. use a different gene, but their gene product functions as a drug efflux pump in the same way as Vga B does.

The inhibiting activity of Vat C can be tested by visualizing the acetylation reaction as described in Allignet et al. (1993) regarding the mechanism of inactivation of A-type compounds conferred by plasmids pIP680 and pIP1156 by thick layer chromatography and NMR.

The inhibiting activity of Vgb B can be tested by detecting the degradation of streptogramin B or a related compound by a microbiological test as described in Allignet et al. (1988).

Plasmids containing the polynucleotides from *Staphylococci*, which confer streptogramin A and/or B resistance, are referred to herein by the following accession numbers:

| Plasmid | Accession No. |
|---|---|
| pIP1714 | I- 1877 |
| pIP1633 | I-1768 |
| pIP680 | I-1767 |

and they have been inserted into vectors which have been deposited at the Collection Nationale de Cultures de Microorganismes ("C.N.C.M.") Institut Pasteur, 28, rue du Docteur Roux, F-75724 Paris Cedex 15, France on June 18, 1997, and August 7, 1996, respectively.

## EXAMPLES

**Example 1**: *Cloning of the vgaB gene carried by plasmid pIP1633*

pIP1633 was isolated from a *S. aureus* transconjugant strain, BM12235, obtained from the donor wild-type *S. aureus* strain, BM3385 (Allignet and El Solh, 1995). This plasmid carried the *vat*B gene located on a 5.5 *Bgl*II fragment, but the other described streptogramin A resistant ($SgA^r$) genes were not detected either by hybridization experiments or by PCR (Allignet and EI Solh, 1995). Since the gene *vga* was carried by all the tested staphylococcal plasmids containing the *vat* gene (Allignet et al., 1996), the presence of a *vga*-related gene was suspected in pIP1633. We therefore searched this gene in the recombinant plasmid, pIP1675 (Fig. 1A), containing the *vatB*-5.5 *Bgl*II fragment of pIP1633.

First, the 2.4 kb *Hin*dIII-*Hae*lll fragment of pIP1675, which contains only 10 nucleotide from *vatB*, was inserted into plasmid pOX300, and the recombinant plasmid, pIP1717 (Fig. 1B), was introduced by electroporation into the *S. aureus* recipient, RN4220 (Kreiswirth et al., 1983). Plasmid pOX300, also named pOX7, (Dyke and Curnock, 1989), is a hybrid of pUCl8 and pEl94ts and replicates in *E. coli* where it confers resistance to ampicillin and to erythromycin, and in *S. aureus* where only resistance to erythromycin is expressed. The *S. aureus* transformants selected on 10$\mu$g/ml erythromycin were resistant to streptogramin A and related compounds (PIIA MICs = 8-16 $\mu$g/ml). Thus, the 2.4-kb *Hin*dIII-*Hae*III insert of pIP1717 (Fig. 1B) probably carried a streptogramin A resistance gene and was sequenced. The nucleotide (nucleotide) sequence of this fragment was determined by the dideoxy method (Sanger et al., 1977) with the reagents and the procedure recommended by the suppliers of the $T^7$ sequencing kit (Pharmacia International). Arrows indicate the direction and extent of each dideoxy-sequencing reaction. (Fig. 1B).

**Example 2**: *The nucleotide sequence of the vgaB gene*

The strategy of sequencing on both strands is outlined in Fig. 1 and the sequence of the 2411-bp *Hin*dIII-*Hae*III insert is given in Fig. 2. An open reading frame (ORF) of 1674 nucleotide extending from nucleotide 682 to 2356 was detected on the same strand as *vatB* (Fig. 2). The 1674 nucleotide ORF contained an ATG start codon at nucleotide 700 to 702 and was preceded by an 8 nucleotide putative RBS. The ΔG (free energy of association) of interaction of the most stable structure between this putative RBS and the 3'-terminus of the 165 rRNA (MacLaughlin et al., 1981; Moran et al., 1982) calculated according to Tinoco et al. (1973) was -79.4 kJ/mol. The sequence located between the ATG codon and the TAA stop codon at nucleotide 2356 to 2358 may encode a 552 amino acid protein of 61,327 daltons (Da). This putative gene, named *vgaB*, had 58.8 % nucleotide identity with the 1572 bp gene, *vga* (Allignet et al., 1992). The G+C content of *vgaB* (27.2 %) is similar to that of *vga* (29 %), but both values are slightly lower than those of the staphylococcal genome (32 to 36 %) (Kloos and Schleifer, 1986). The nucleotide sequence of *vgaB* has been submitted to the GenBank/EMBL data bank under accession no. u82085.

Example 3: *Amino acid sequence analysis of VgaB*

The predicted translation product of the *vgaB* gene, VgaB, has a calculated isoelectric point (pI) of 9.60. The hydropathy plot of the VgaB sequence according to the algorithm of Kyte and Doolittle (1982) indicates the protein to be hydrophilic. No similarity to known signal sequences of secreted proteins (von Heijne, 1986; Watson, 1984) was observed.

The amino acid sequence of VgaB was compared with the sequences available in databases (GenBank, release 97.0; EMBL, release 48; SwissProt, release 34). Significant similarity to the ATP-binding domains of numerous ATP-binding Cassette (ABC) proteins was found. The protein giving the best match was Vga (48.3 % identical amino acid, 70.4 % similar amino acid). VgaB and Vga each contain two ATP-binding domains sharing 38.8 % and 39.1 % identical amino acid, respectively. Each of these domains

includes the two ATP-binding motifs described by Walker et al. (1982) (Fig.2). Moreover, the highly conserved SGG sequence of loop 3 found between the two ATP-binding motifs of all investigated ATP-binding proteins (Barrasa et al., 1995; Hyde et al., 1990) was detected in Vga (Allignet et al., 1992) and VgaB (Fig. 2). According to the predicted tertiary structure of ABC model cassette, this loop would be conveniently located to interact with the cell membrane (Hyde et al., 1990). The inter-ATP-binding domain of VgaB is more rich in glutamine (11 Q in 155 amino acid total) than the rest of the sequence of the protein (11 Q/397 amino acid). In contrast, the proportion of glutamine in the inter-ATP-binding domain of Vga is similar to that in the other part of the protein (4 Q/156 amino acid and 14 Q/366 amino acid, respectively). Neither Vga nor VgaB contains hydrophobic transmembrane domains.

The ABC protein MsrA (Ross et al., 1990) is the most similar to Vga and VgaB (35.2 % and 34.4 % identical amino acid, respectively). MsrA confers resistance to erythromycin by increasing the efflux of this antibiotic and to streptogramin B by a mechanism not yet elucidated. MsrA contains two ATP-binding domains with 31.8% amino acid identity and separated by a Q-linker, but no hydrophobic stretches that might be potential membrane spanning domains. The hydrophobic proteins, which are expected to interact with MsrA, are those encoded by similar genes mapping near MsrA in two staphylococcal strains (*smpA*, *smpB*) and also those on the chromosome of the *S. aureus* recipient strain, RN4220 (*smpC*), which does not carry *msrA* (Ross et al., 1995). Ross et al. (1996) have recently reported that SmpC found in the chromosome of RN4220 is not essential for the expression of resistance to erythromycin conferred by MsrA. Thus, further experiments are required to elucidate the mechanisms of resistance conferred by *msrA*, *vga*, or *vgaB* genes.

Several ABC transporters, which do not have alternating hydrophobic domains, have been grouped in a subfamily in order to distinguish them from the members of the $ABC_2$ transporter subfamily, the members of which contain hydrophobic transmembrane domains (Barrasa et al., 1995; Olano et al., 1995; Peschke et al., 1995). Thus, VgaB may

be considered as a new member of the former ABC transporter subfamily. Excluding VgaB, Vga, and MsrA, most of the known ABC transporters that contain two ATP-binding cassettes but no hydrophobic domain(s) were found in lantibiotic or antibiotic producing microorganisms in which they are involved in the active excretion of these molecules. These transporters are encoded by the following genes: *ard1*, an amino-acylnucleoside antibiotic resistance gene from *Streptomyces capreolus* (Barrasa et al., 1995); *carA*, a carbomycin-resistance gene from *Streptomyces thermotolerans* (Schoner et al., 1992); *lmrC*, a lincomycin-resistance gene from *Streptomyces lincolnensis* (Peschke et al., 1995); *oleB*, an oleandomycin-resistance gene from *Streptomyces antibioticus* (Olano et al., 1995); *srmB*, a spiramycin-resistance gene from *Streptomyces ambofaciens* (Geistlich et al., 1992); *tlrC*, a tylosin-resistance gene from *Streptomyces fradiae* (Rosteck et al., 1991); and *petT*, a pep5 epidermin-resistance gene from *Staphylococcus epidermidis* (Meyer et al., 1995). The amino acid identity between each of these latter ABC transporters and VgaB is between 23.6 % and 28.7 %.

Degenerate primers designed from an analysis of the alignment of the amino acid sequence of Vga and VgaB may be helpful to detect such putative genes by PCR experiments. In the streptogramins producers, the described resistance to these antibiotics consists of streptogramin A inactivation by an as yet unknown mechanism (Fierro et al., 1989), streptogramin B inactivation by a lactonase (Kim et al., 1974) and putative increased export of streptogramin A and streptogramin B by an integral membrane protein, Ptr, exploiting transmembrane proton gradients (Blanc et al., 1995). The NMR spectra of the modified A compounds may be analyzed to verify if their inactivation in the antibiotic producers is similar to that due to the proteins Vat or VatB, which transfer an o-acetyl group to position C14 of PIIA (Allignet et al., 1993). Interestingly, the staphylococcal gene *vgb* (Allignet et al., 1988) found in most plasmids carrying *vga* and *vat* (Allignet et al., 1996), encodes a protein inactivating streptogramin B and related compounds by cleavage of the lactone ring.

**Example 4**: *Distribution and location of the vgaB gene in 52 SgA$^R$ and independent wild-type staphylococci*

A recombinant plasmid containing a fragment of *vgaB*, pIP1705, was constructed to serve as a probe in hybridization experiments under stringent conditions as described previously (Allignet et al., 1996). pIP1705 consists of pUC19 cleaved with *Sal*I and *Pst*I, and an insert of 1051 bp amplified from within *vgaB* by the following primers, which introduce *Pst*I or *Sal*I sites:

**Oligo I** 5'-AA**GTCGAC**TGACAATATGAGTGGTGG-3'
*Sal*I

**Oligo II** 5'-**CTGCAG**ATGCCTCAACAGCATCGATATCC-3'
*Pst*I

The 52 SgA$^r$ staphylococci investigated (Allignet et al., 1996; El Solh et al., 1980; Loncle et al., 1993) included 10 strains (7*S. aureus*, 1*S. simulans*, 1*S. haemolyticus*, and 1*S. cohnii urealyticum*), which harbored 26 to 45 kb plasmids containing *vga*, *vat*, and *vgb*; 21 strains (20 *S. aureus* and one *S. epidermidis*), which harbored 50 to 90 kb plasmids containing *vatB*; 16 strains (12 *S. epidermidis*, three *S. haemolyticus* and one *S. aureus*) with 6 to 15 kb plasmids containing *vga*; one *S. epidermidis* strain which harbored a plasmid of approximately 20 kb containing *vga-vat*; and four *S. aureus* strains, which do not carry nucleotide sequences hybridizing with *vat*, *vatB*, *vga*, or *vgb*. Nucleotide sequences hybridizing with pIP1705 were found only in the 21 large plasmids containing *vatB*. In all these 21 plasmids including pIP1633, the hybridizing nucleotide sequences were detected on a 1.5 kb *Eco*RI fragment, which also hybridized with *vatB*, suggesting that *vgaB* and *vatB* have conserved relative positions.

**Example 5**: *Results concerning vatC and vgbB genes*

The *Staphylococcus cohnii* strain, BM10711, resistant to the synergistic mixtures streptogramin A and streptogramin B and related compounds (pristinamycin, virginiamycin, synergistin, mikamycin, Quinupristin-Dalfopristin) was analyzed. This strain was isolated at Douera hospital (Algeria) where the pristinamycin was frequently used topically. The strain was isolated (Liassin et al., 1997) from a sample provided from a cupboard located in a room occupied by patients suffering from chronic osteomyelitis.

The strain BM10711 harbored several plasmids including pIP1714 (5kb). This plasmid was isolated by electroporation in a *S. aureus* recipient strain, RN4220. The transformant, harboring pIP1714, was selected on BHIA containing 10 µg/ml pristinamycin IIA. Plasmid pIP1714 conferred resistances to streptogramin A and streptogramin B and related compounds.

Plasmid pIP1714 was linearized by cleavage with *Hind*III and cloned in the *Hind*III site of the vector pOX7 also named pOX300 (Dyke et al., 1989, FEMS Microbiol. Lett. 58:209-216). pOX7 results from the cointegration of the *E. coli* vector, pUC18, and *S. aureus* plasmid, pE194. The recombinant plasmid pIP1715 consisting of pOX7 and pIP1714 was used to sequence pIP1714 in its entirety. The gene *vatC* (636 nucleotides) encoding an acetyltransferase inactiving streptogramin A and related compounds and the gene vgbB (885 nucleotides) encoding a lactonase inactiving streptogramin B and related compounds were found to be carried by this plasmid. The gene *vatC* had 71.7, 62.2 and 64.1 % nucleotides identity with *vat*-related gene, *vatB* and *satA* respectively and the gene *vgbB* presents 69.5 % nucleotides identity with the gene *vgb*.

VatC acetyltransferase exhibits significant similarity with acetyltransferases having the same enzymatic activity and encoded by the genes *vatC*, *vatB*, and *sat* (respectively 69.8,58.2 and 66.0 % amino acids identity). These proteins belong to a family of xenobiotic acetyltransferases modifying various substrates including streptogramin A and

related antibiotics. VgbB lactonase exhibits as well significant similarity with Vgb inactivating streptogramin B and related (67.0 % amino acids identity).

The two other genes carried by pIP1714 are *pre* and *repB*, encoding proteins involved in mobilization and replication, respectively. These two genes are homologous to those carried by the staphylococcal plasmid, pUB110 (McKenzie et al., 1986, Plasmid 15:93-103). Moreover, as reported in Figure 5, the intergenic sequences of pIP1714 delimited by *vatC* and *repB* also exhibited significant similarities with pUB110.

Example 6: *Plasmid DNA isolation from PIIA$^R$ staphylococci*

The staphylococci were grown after overnight incubation at 37°C in 200 ml BHI containing 10 µg/ml of PIIA. After 15 min centrifugation at 8000 rpm, the pellet was resuspended in 25 ml TES (Tris 50 mM, EDTA 1 mM, saccharose 7%). After adding 150 µg of lysostaphin, the mixture was incubated 30 min at 37°C. Then, 2ml of SDS 20% and 6 ml of EDTA 0.25 M were added and the suspension was incubated 15 min at 37°C. 8 ml of NaCl 5M were added and the mixture was kept 90 min at +4°C. After 30 min centrifugation at 8000 rpm, the supernatent was incubated 15 min at 37°C with 5 µg of Rnase (Boehringer). 10 µg of Proteinase K were added and the suspension was incubated 15 min at 65°C. DNA was precipitated using isopropanol (0.6 V for 1 V of DNA solution). After 30 min centrifugation at 8000 g, the pellet was washed with 10 ml ethanol 70%. The washed DNA was dried at 56°C, dissolved in 10 ml water and purified by dye-buoyant density centrifugation (ethidium bromide - cesium chloride). The extrachromosomal band was collected. After removing ethidium bromide, the solution of plasmid DNA was dialysed using TE buffer (Tris, 10 mM, EDTA 1 mM, pH 7).

Example 7: *Plasmid DNA isolation from E. coli*

Cf. QIAfilter plasmid maxi protocol for large-scale preparations and QIAprep Spin plasmid kit protocol for mini-preparations.

Quiagen GmbH and Quiagen Inc. (Hilden, Germany)

- Plasmid maxi kit Ref : 12262
- Miniprep kit Ref : 27104

**Example 8**: *Transformation by electroporation of the S. aureus recipient strain, RN4220*

1 - Preparation of cells

200ml of BHI was inoculated with 20ml of an overnight culture of RN4220 *(Kreiswirth et al., Nature 1983, 306:709-712)* and incubated at 37°C with shaking. When the OD reached 0.4 at 600 nm, the suspension was kept in ice. The pellet was washed three times with 20 ml of cold Hepes buffer (saccharose 9.31 % - Hepes 0.19 % - pH. 7.4). The pellet was resuspended in 2.5 ml of Hepes buffer containing 10% glycerol. Aliquots of 100 µl cell suspension ($3.10^{10}$/ml) were stored at -80°C.

2 - Electroporation

After thawing at room temperature, the 100 µl aliquot of cells was kept in ice. After adding 10 µl of a solution containing 1 µg of plasmid DNA, the mixture was transferred to a cold 0.2 cm electroporation cuvette. The Gene Pulser (BioRad) was set at 25 uF and 2.5 KV and the Pulse Controller to 100Ω. This produced a pulse with a constant time of 2.3 to 2.5 m sec. The cuvette was removed from the chamber and 1 ml of SOC (2% bactotryptone, 0.5% bactoyeast extract, 10mM NaCl, 2.5mMKCl, 10mM $MgCl_2$, 10mM $MgSO_4$, 20 mM glucose) was added. The cell suspension was transferred in a propylene tube and incubated with shaking at 37°C for 1 hr. The suspension was then plated on selective medium, which consisted of BHIA containing 10 µg/ml erythromycin or 10

µg/ml of PIIA. The plates were incubated 48 h at 37°C and the transformants isolated on selective medium. The further studies were carried out on a single isolated colony.

**Example 9**: *Polymerase chain reaction*

DNA was amplified by PCR in a Crocodile II thermal cycler (Appligène) with approximately 10ng of cellular DNA or 1ng of plasmid DNA. The reaction mixture contained 0.6 µM of each oligonucleotide serving as primer, 200 µM of each deoxynucleotide triphosphate, 2.5 U of *Taq* DNA Polymerase (Amersham, Int.), and 1 x buffer (Amersham, Int.). The final reaction volume was adjusted to 100 µl with $H_2O$ and the sample was then covered by 50 µl of heavy white mineral oil (Sigma Chemical Co, St. Louis, Missouri).

PCR experiments were carried out at high or low stringency, depending on the primers used. At high stringency, the PCR was performed with a precycle of 3 min at 95°C and 2 min at 60°C, 30 cycles of 20 sec at 72°C, 20 sec at 95°C, 20 sec at 60°C followed by a cycle of 1 min at 72°C. At low stringency, the PCR was performed with a precycle of 5 min at 95°C, 35 cycles of 2 min at 40°C, 1 min 30 sec at 72°C, 30 sec at 95°C followed by a cycle of 4 min at 40°C and 12 min at 72°C. The oligonucleotides used at high stringency are indicated in the Table below.

| | PRIMER |
|---|---|
| *vgaB* | **Oligo I** 5'-AA<u>GTCGAC</u>TGACAATATGAGTGGTGG-3'<br>*Sal*I<br>**Oligo II** 5'-<u>CTGCAG</u>ATGCCTCAACAGCATCGATATCC-3'<br>PstI |
| *vatC* | **Oligo III** 5'- AT<u>GAATTC</u>GCAAATCAGCAAGG -3'<br>*Eco*RI<br>**Oligo IV** 5'- TCGTCTC<u>GAGCTC</u>TAGGTCC -3'<br>*Sac*I |
| *vgbB* | **Oligo V** 5'- CAGCAG<u>TCTAGA</u>TCAGAGTGG -3'<br>*Xba*I<br>**Oligo VI** 5'- CATAC<u>GGATCC</u>ACCTTTTCC -3'<br>*Bam*H1 |

**Example 10**: *Labelling of DNA probes*

Plasmid DNA was labelled with [$\alpha$-$^{32}$P]dCTP (110 Tbq mmol$^{-1}$) by the random printing technique using the Megaprime DNA labelling system (Amersham).

**Example 11**: *Blotting and hybridization*

Hybond-N+membranes (Amersham) were used for blotting. DNA was transferred from agarose gels to the membranes by the capillary blotting method of Southern Blotting. DNA was denatured and fixed to the membranes according to the protocol described in the handbook user of Hybond-N+ membranes.

Prehybridization and hybridization were done at 68°C in a mixture containing 5X SSPE (1X SSPE is 0.3 M NaCl, 30 m tri-sodium citrate), 5X Denhardt's solution, 0.5% (w/v) SDS, and 100 µg $ml^{-1}$ salmon sperm DNA. The membranes containing DNA transferred from agarose gels were treated with 10 ng $ml^{-1}$ radiolabeled DNA probe. Washing was started with two successive immersions in 2X SSPE, 0.1% SDS, at room temperature for 10 min, followed by one immersion in 1X SSPE, 0.1% SDS, at 68°C for 15 min, and finally by one immersion in 0.1 X SSPE, 0.1% SDS, at 68°C for 15 min. The washed blots treated with the radiolabeled probe were exposed to Fuji RX film at -70°C.

Example 12: *Nucleotides sequence determination*

**For *vatC* and *vgbB***, the sequencing reaction was performed by PCR amplification in a final volume of 20 µl using 500 ng of plasmid DNA, 5-10 pmoles of primer and 9.5 µl of DyeTerminators premix according to Applied Biosystems protocol. After heating to 94°C for 2 min, the reaction was cycled as the following: 25 cycles of 30s at 94°C, 30s at 55°C, and 4 min at 60°C (9600 thermal cycler Perkin Elmer). Removal of excess of DyeTerminators were performed using Quick Spin columns (Boehringer Mannheim). The samples were dried in a vacuum centrifuge and dissolved with 4µl of deionized formamide EDTA pH 8.0 (5/1). The samples were loaded onto an Applied Biosystems 373A sequencer and run for 12 h on a 4.5% denaturing acrylamide gel.

- Primers used for sequencing the following genes:

- *vatC*

| | |
|---|---|
| 5'-GAAATGGTTGGGAGAAGCATACC-3' | 5'-CAGCAATCGCGCCCGTTTG-3' |
| 5'-AATCGGCAGAATTACAAACG-3' | 5'-CGTTCCCAATTTCCGTGTTACC-3' |

- *vgbB*

| | |
|---|---|
| 5'-GTTTCTATGCTGATCTGAATC-3' | 5'-GTCGTTTGTAATTCTGCCGATT-3' |
| 5'-GGTCTAAATGGCGATATATGG-3' | 5'-TTCGAATTCTTTTATCCTACC-3' |

For *vgaB*. DNA was sequenced according to the instructions provided by the T7SequencingTm kit from Pharmacia Biotech (Uppsala, Sweden), procedures C and D.

- Primers used for sequencing the following genes:

- *vgaB*

| | |
|---|---|
| 5'-GCTTGGCAAAAGCAACC-3' | 5'-TGAATATAGGATAG-3' |
| 5'-TTGGATCAGGGCC-3' | 5'-CAATTAGAAGAACCAC-3' |
| 5'-CAATTGTTCAGCTAGG-3' | 5'-GAATTCATTCTATGG-3' |
| 5'-TACACCATTGTTACC-3' | 5'-CAAGGAATGATTAAGCC-3' |
| 5'-GATTCAGATGTTCCC-3' | 5'-TCATGGTCGCAATG-3' |
| 5'-GTTGCTTTCGTAGAAGC-3' | 5'-GTTATGTCATCCTC-3' |
| 5'-GGTTCATCTACGAGC-3' | 5'-GGATATCGATGCTG-3' |
| 5'-GCCAACTCCATTC-3' | 5'-CCTAGCTGAACAATTG-3' |
| 5'-GAAGGTGCCTGATCC-3' | 5'-ATACTAGAAATGC-3' |

**Example 13**: *DNA cloning*

A standard protocol was followed for cloning into the vector p0X7, also named pOX300, the 2.4 kb *Hind*fII-*Hae*III fragment of plP1633 carrying *vgaB* (Fig. 1) and the plasmid plP1714 carrying *vatC* and *vgbB* (Fig. 4), linearized by cleavage with *Hind*III. The vector DNA (10-20 µg) and the plasmids used in cloning experiments were cleaved with the appropriate restriction enzymes (30 Units) and purified by GeneClean Kit (Bio 101, La Jolla, Calif.). To avoid religation, the vector cleaved with a single enzyme was dephosphorylated by 30 min incubation at 37°C with 5 Units of alcaline phosphatase. Ligation was carried out in a total reaction volume of 10 µl containing 0.1 µg of the vector, 0.1 µg of the plasmid, 0.5 mM ATP, 1 X T4 DNA ligase buffer and 0.1 Weiss Unit of T4 DNA ligase. After overnight incubation at 16°C, 1 to 2 µl of the ligation mixture are used for transforming competent *E. coli* and the transformants were selected on solid media containing 100 µg/ml of ampicillin.

**Example 14**: *Susceptibility to antimicrobial agents*

Susceptibility to antimicrobial agents was determined with a disk diffusion assay and commercially available disks (Diagnostic Pasteur). Additional disks prepared in our laboratory contained streptogramin A (20 µg) or streptogramin B (40 µg).

- NCCLS: Performance standards for antimicrobial disk susceptibility test, 1984, Approved standard M2-A3, 4:369-402.
- ECCLS: Standard for antimicrobial susceptibility testing by diffusion methods, 1985, ECCLS Document, 5:4-14.

Minimal inhibitory concentrations (MICs) of antibiotics were determined by serial twofold dilutions of antibiotics in MHA (Ericson H.M. and S.C. Sherris, ActaPathol. Microbiol. Scand., 1971, Suppl. 217:Section B).

Despite the relatively low frequency of detection of $SgA^R$ staphylococci (1-10%) (Loncle et al., 1993; Allignet et al., 1996), four genes encoding resistance to streptogramin A have been detected and other resistance gene(s) are suspected to be carried by staphylococci. Surprisingly, the present and previous studies (Allignet et al., 1996) indicate that staphylococcal plasmids carrying two genes encoding streptogramin A resistance by two distinct mechanisms (inactivation by acetyltransferases and increased efflux) are widespread among staphylococci (32 of the 48 plasmids investigated).

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WHAT IS CLAIMED IS:

1. A purified polynucleotide comprising the nucleotide sequence selected from the group consisting of SEQ ID NO: 1 or a fragment derived from SEQ ID NO: 1 containing 15 to 40 nucleotides, SEQ ID NO: 11, and SEQ ID NO: 12.

2. A purified peptide comprising the amino acid sequence encoded by the nucleotide sequence selected from the group consisting of SEQ ID NO: 1 or a fragment derived from SEQ ID NO: 1 containing 15 to 40 nucleotides, SEQ ID NO: 11, and SEQ ID NO: 12.

3. A purified peptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 4 or a fragment derived from SEQ ID NO: 4 containing at least 10 amino acids, SEQ ID NO: 7, and SEQ ID NO: 8.

4. A purified polynucleotide comprising the nucleotide sequence of SEQ ID NO: 3 or a fragment derived from SEQ ID NO: 3 containing 15 to 40 nucleotides.

5. A purified peptide comprising the amino acid sequence encoded by the nucleotide sequence selected of SEQ ID NO: 3 or a fragment derived from SEQ ID NO: 3 containing 15 to 40 nucleotides.

6. A purified peptide comprising the amino acid sequence of SEQ ID NO: 6 or a fragment derived from SEQ ID NO: 6 containing at least 10 amino acids.

7. A purified polynucleotide comprising the nucleotide sequence selected from the group consisting of SEQ ID NO: 2 or a fragment derived from SEQ ID NO: 2 containing 15 to 40 nucleotides, SEQ ID NO: 13, and SEQ ID NO: 14.

8. A purified peptide comprising the amino acid sequence encoded by the nucleotide sequence selected from the group consisting of SEQ ID NO: 2 or a fragment derived from SEQ ID NO: 2 containing 15 to 40 nucleotides, SEQ ID NO: 13, and SEQ ID NO: 14.

9. A purified peptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 5 or a fragment derived from SEQ ID NO: 6 containing at least 10 amino acids, SEQ ID NO: 9, and SEQ ID NO: 10.

10. A composition of polynucleotide sequences useful for the detection of resistance to streptogramins A and/or B and related compounds comprising at least a nucleotide sequence selected from the group consisting of the polynucleotide or gene or cDNA po*vga*B, *vgb*B, and *vat*C .

11. A composition of polynucleotide sequences useful for the detection of resistance to streptogramin A or streptogramin B and related compounds comprising at least an amino acid sequence from a polypeptide encoded by a polynucleotide selected from the group consisting of the polynucleotide, gene, or cDNA *vga*B, *vgb*B, and *vat*C

12. A composition of polynucleotide sequences encoding resistance to streptogramins and related compounds, or inducing streptogramin resistance in Gram-positive bacteria, wherein said composition comprises at least a nucleotide sequence encoding a molecule containing ATP binding motifs conferring resistance to streptogramin A and related compounds and at least a nucleotide sequence selected from the following sequences:

a) a nucleotide sequence encoding an acetyltransferase conferring resistance to streptogramin A and related compounds; and

b) a nucleotide sequence encoding a lactonase conferring resistance to streptogramin B and related compounds.

13. A composition of polynucleotide sequences encoding resistance to streptogramins and related compounds, or inducing streptogramin resistance in Gram-positive bacteria, wherein said composition comprises at least a nucleotide sequence encoding an acetyltransferase conferring resistance to streptogramin A and related compounds and at least a nucleotide sequence selected from the following sequences:

a) a nucleotide sequence encoding a molecule containing ATP binding motifs conferring resistance to streptogramin A and related compounds; and

b) a nucleotide sequence encoding a lactonase conferring resistance to streptogramin B and related compounds.

14. A composition of polynucleotide sequences encoding resistance to streptogramins and related compounds, or inducing streptogramin resistance in particular in Gram-positive bacteria, wherein said composition comprises at least a nucleotide sequence encoding a lactonase conferring resistance to streptogramin B and related compounds and at least a nucleotide sequence selected from the following sequences:

a) a nucleotide sequence encoding a material containing ATP binding motifs conferring resistance to streptogramin A and related compounds; and

b) a nucleotide sequence encoding an acetyltransferase conferring resistance to streptogramin A and related compounds.

15. A composition of polynucleotide sequences according to any one of the claims 12 to 14, wherein the polynucleotide sequence encoding a molecule containing ATP binding motifs confers resistance to streptogramin A and related compounds in *Staphylococcus*, and wherein the polynucleotide corresponds to a *vga*B nucleotide sequence represented in the SEQ ID NO: 1 or to a nucleotide sequence having at least 70 % of identity with the *vga*B complete nucleotide sequence, or corresponds to a polynucleotide hybridizing with said SEQ ID NO: 1 under stringent conditions, or to a fragment containing between 20 and 30 nucleotides of SEQ ID NO: 11 or SEQ ID NO: 12 or, wherein the polynucleotide sequence encodes a polypeptide having at least 60 % homology with the complete SEQ ID NO: 4 or 80 % with SEQ ID NO: 11 or SEQ ID NO: 12.

16. A composition of claim 15, wherein the polynucleotide sequence encoding a molecule containing ATP binding motifs confers resistance to streptogramin A and related compounds in *Staphylococcus aureus*.

17. A composition of polynucleotide sequences according to any one of the claims 12 to 14, wherein the polynucleotide sequence encoding an acetyltransferase confers resistance to streptogramin A and related compounds in *Staphylococcus*, and wherein the polynucleotide corresponds to a *vat*C nucleotide sequence represented in the SEQ ID NO: 2 or to a nucleotide sequence having at least 70 % of identity with the *vat*C complete nucleotide sequence, or corresponds to a polynucleotide hybridizing with said SEQ ID NO: 2 under stringent conditions or to a fragment of SEQ ID NO: 2 containing between 20 and 30 nucleotides, or to SEQ ID NO: 13 or SEQ ID NO: 14, or wherein the polynucleotide sequence encodes a polypeptide having at least 60 % homology with the complete SEQ ID NO: 5 or 80 % with SEQ ID NO: 13 or SEQ ID NO: 14.

18. A composition of claim 17, wherein the polynucleotide sequence encoding an acetyltransferase confers resistance to streptogramin A and related compounds in *Staphylococcus cohnii*.

19. A composition of polynucleotide sequences according to any one of the claims 12 to 14, wherein the polynucleotide sequence encoding a lactonase confers resistance to streptogramin B and related compounds in *Staphylococcus*, and wherein the polypeptide corresponds to a *vgb*B nucleotide sequence represented in the SEQ ID NO: 3 or to a nucleotide sequence having at least 70 % of identity with the *vgb*B complete nucleotide sequence, or corresponds to a polynucleotide hybridizing with said SEQ ID NO: 3 under stringent conditions or to a fragment of said SEQ ID NO: 3 containing 20 to 40 nucleotides, or wherein the polynucleotide sequence encodes a polypeptide having at least 60 % homology with the complete SEQ ID NO: 6 .

20. A composition of claim 19, wherein the polynucleotide sequence encoding a lactonase confers resistance to streptogramin A and related compounds in *Staphylococcus cohnii*.

21. A composition of polynucleotide sequences according to claim 12 or 13, wherein said composition comprises at least one nucleotide sequence encoding a molecule containing ATP binding motifs conferring resistance to streptogramin A and related

compounds and at least one nucleotide sequence encoding an acetyltransferase conferring resistance to streptogramin A and related compounds.

22. A composition of polynucleotide sequences according to claim 21, wherein said composition comprises at least a *vga*B nucleotide sequence encoding a molecule containing ATP binding motifs conferring resistance to streptogramin A and related compounds and at least a *vat*B nucleotide sequence encoding an acetyltransferase conferring resistance to streptogramin A and related compounds.

23. A purified polynucleotide that hybridizes specifically under stringent conditions with a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, and SEQ ID NO: 14.

24. A polynucleotide fragment comprising at least 10 nucleotides, that hybridizes under stringent conditions with a sequence according to any one of claims 1- 9 or 23 or the polynucleotide complementary fragment thereof.

25. A polynucleotide fragment of claim 12, wherein said fragment corresponds to at least one of the following sequences:

- Oligo I 5'-AAGTCGACTGACAATATGAGTGGTGG-3'
- Oligo II 5'-CTGCAGATGCCTCAACAGCATCGATATCC-3'.

26. A polynucleotide fragment of claim 12, wherein said fragment corresponds to at least one of the following sequences:

- Oligo III 5'- ATGAATTCGCAAATCAGCAAGG -3'
- Oligo IV 5'- TCGTCTCGAGCTCTAGGTCC -3'.

27. A polynucleotide fragment of claim 12, wherein said fragment corresponds to at least one of the following sequences:

- Oligo V 5'- CAGCAGTCTAGATCAGAGTGG -3'
- Oligo VI 5'- CATACGGATCCACCTTTTCC -3'.

28. A recombinant DNA sequence comprising at least a nucleotide sequence according to any one of claims 1, 4, or 7 under the control of regulatory elements that

regulate the expression of resistance to antibiotics of the streptogramin family in a defined host.

29. A recombinant vector comprising the DNA sequence of claim 31, wherein the vector comprises a plasmid pIP1633 deposited with the C.N.C.M. under the Accession No. I-1768 or a plasmid pIP1714 deposited with the C.N.C.M. under the number I-1877 on June 18, 1997.

30. A recombinant cell host comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 3, SEQ ID NO: 2, SEQ ID NO: 13, and SEQ ID NO: 14, or the recombinant vector of claim 32.

31. A purified polypeptide or a peptide fragment having at least 10 amino acids, which is recognized by antibodies directed against a polynucleotide sequence conferring resistance to streptogramin and related compounds, corresponding to a polynucleotide sequence according to any one of claims 1, 4, or 7.

32. A polynucleotide comprising the full length coding sequence of a *Staphylococcus* streptogramin A and/or B resistant gene containing a polynucleotide sequence according to any one of claims 1, 4, or 7.

33. A monoclonal or polyclonal antibody directed against a polypeptide or a peptide fragment encoded by the polynucleotide sequences according to any one of claims 1, 4, or 7.

34. A method of detecting the presence of bacterium harboring the polynucleotide sequences according to any one of claims 1, 4, or 7 in a biological sample comprising:

a) contacting bacterial DNA of the biological sample with a primer or a probe according to any one of claims 24-27, which hybridizes with a nucleotide sequence encoding resistance to streptogramins;

b) amplifying the nucleotide sequence using said primer or said probe; and

c) detecting the hybridized complex formed between said primer or probe with the DNA.

35. A kit for detecting the presence of bacterium having resistance to streptogramin A and/or streptogramin B and harboring the polynucleotide sequences according to any one of the claims 1, 4, or 7 in a biological sample, said kit comprising:

a) a polynucleotide probe according to any one of claims 24-27; and

b) reagents necessary to perform a nucleic acid hybridization reaction.

36. A kit for detecting the presence of bacterium having resistance to streptogramin A and harboring the polynucleotide sequences according to claim 1 or claim 7 in a biological sample, said kit comprising:

a) a polynucleotide probe according to claim 24-26; and

b) reagents necessary to perform a nucleic acid hybridization reaction.

37. A method of screening active antibiotics for the treatment of the infections due to Gram-positive bacteria, comprising the steps of:

a) bringing into contact a Gram-positive bacteria having a resistance to streptogramin A or streptogramin B and related compounds and containing the polynucleotide sequences according to any one of the claims 1, 4, or 7 with the antibiotic; and

b) measuring an activity of the antibiotic on the bacteria having a resistance to streptogramins and related compounds.

38. A method of screening for active synthetic molecules capable of penetrating into a bacteria of the family of staphylococci, wherein the inhibiting activity of these

molecules is tested on at least a polypeptide encoded by the polynucleotide sequences according to any one of claims 1, 4, or 7 comprising the steps of:

a) contacting a sample of said active molecules with the bacteria;

b) testing the capacity of the active molecules to penetrate into the bacteria and the capacity of inhibiting a bacterial culture at various concentration of the molecules; and

c) choosing the active molecule that provides an inhibitory effect of at least 80% on the bacterial culture compared to an untreated culture.

39. An *in vitro* method of screening for active molecules capable of inhibiting a polypeptide encoded by the polynucleotide sequences according to any one of claims 1, 4, or 7, wherein the inhibiting activity of these molecules is tested on at least said polypeptide, said method comprising the steps of:

a) extracting a purified polypeptide according to claim 31;

b) contacting the active molecules with said purified polypeptide;

c) testing the capacity of the active molecules, at various concentrations, to inhibit the activity of the purified polypeptide; and

d) choosing the active molecule that provides an inhibitory effect of at least 80 % on the activity of the said purified polypeptide.

40. A composition of a polynucleotide sequence encoding resistance to streptogramins and related compounds, or inducing resistance in Gram-positive bacteria, wherein said composition comprises a nucleotide sequence corresponding to the resistance phenotype of the plasmid pIP1633 deposited with the C.N.C.M. under the Accession No. I-1768 and of the plasmid pIP1680 deposited with the C.N.C.M. under the Accession No. I-1767 and of the plasmid pIP1714 deposited with the C.N.C.M. under the number I-1877 on June 18, 1997.

41. A method of detecting the presence of bacterium harboring the polynucleotide sequences according to claim 1 or claim 7 in a biological sample, said method comprising the steps of:

a) contacting said sample with an antibody according to claim 36 that recognizes a polypeptide encoded by said polynucleotide sequences; and

b) detecting said complex.

42. A diagnostic kit for *in vitro* detecting the presence of bacterium harboring the polynucleotide sequences according to claim 1 or claim 7 in a biological sample, said kit comprising:

a) a predetermined quantity of monoclonal or polyclonal antibodies according to claim 33;

b) reagents necessary to perform an immunological reaction between the antibodies and a polypeptide encoded by said polynucleotide sequences; and

c) reagents necessary for detecting said complex between the antibodies and the polypeptide encoded by said polynucleotide sequences.

# INTERNATIONAL SEARCH REPORT

Intr ional Application No
PCT/IB 98/00962

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/54 C12N9/10 C12N15/31 C07K14/31 C12N15/55
C12N9/18 C12N15/74 C12N1/21 C12Q1/14 C12Q1/34
C12Q1/48 C12Q1/68 G01N33/569 C07K16/12 C07K16/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | ALLIGNET J. ET AL.: "Sequence of a staphylococcal plasmid gene, vga, encoding a putative ATP-binding protein involved in resistance to virginiamycin A-like antibiotics" GENE, vol. 117, 1992, pages 45-51, XP002083993 cited in the application see the whole document | 1-3, 12-16, 23,24, 28, 31-33, 37-42 |
| Y | | 21,22 |
| A | | 34-36 |

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 17 November 1998 | 01.03.99 |
| Name and mailing address of the ISA<br>European Patent Office, P.B. 5818 Patentlaan 2 | Authorized officer |

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 98/00962

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 //(C12N1/21,1:19,1:445)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | ALLIGNET J. ET AL.: "Sequence of a staphylococcal gene, vat, encoding an acetyltransferase inactivating the A-type compounds of virginiamycin-like antibiotics" GENE, vol. 130, 1993, pages 91-98, XP002083994 cited in the application see page 92, left-hand column, paragraph 1 see page 93; figure 2 --- -/-- | 21,29, 34-36,40 |
| Y | | 21 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 17 November 1998 | |
| Name and mailing address of the ISA<br>European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk | Authorized officer |

# INTERNATIONAL SEARCH REPORT

Inter "onal Application No
PC1/IB 98/00962

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | ALLIGNET J. AND EL SOLH N.: "Diversity among the Gram-positive acetyltransferases inactivating Streptogramin A and structurally related compounds and characterization of a new staphylococcal determinant, vatB" ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 39, no. 9, September 1995, pages 2027-2036, XP002084725 cited in the application | 29,40 |
| Y | see page 2027 see page 2029, right-hand column, paragraph 5 | 22 |
| | --- | |
| X | WO 96 08582 A (BERGERON MICHEL G.; OUELLETTE MARC; ROY PAUL H. (CA)) 21 March 1996 see abstract see page 17, line 6-32 see page 38; table 8 Seq.ID:176 see page 151 - page 152 | 1,2, 12-16, 21,23, 24,32, 34-36 |
| | --- | |
| P,X | ALLIGNET J. AND EL SOLH N.: "Characterization of a new staphylococcal gene, vgaB, encoding a putative ABC transporter coferring resistance to streptogramin A and related compounds" GENE, vol. 202, no. 1/2, 20 November 1997, pages 133-138, XP002083995 see the whole document | 1-3, 10-16, 21-25, 28-32, 34-36,40 |
| | --- | |

# INTERNATIONAL SEARCH REPORT

national application No.
PCT/IB 98/00962

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

See Further Information sheet enclosed.

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-3, 15, 16, 25 all totally; 10-14, 21-24, 28-42 all partially.

   Polynucleotide comprising the nucleotide sequence as in Seq.ID:1, fragments, hybridizing sequences and complementary sequences thereof. Corresponding polypeptide comprising the aminoacid sequence as in Seq.ID:4 or fragments thereof. Recombinant vector comprising said DNA sequence, recombinant host cell comprising said polynucleotide. Antibody directed against said polypeptide. Application of said polynucleotide or of said antibody in diagnostics, kits thereof. Methods of screening involving bacteria containing said polynucleotide, or said polypeptide.

2. Claims: 4-6, 19, 20, 27 all totally; 9-14, 23, 24, 28-35, 37-40 all partially.

   Idem as invention 1 but concerning Seq.ID:3 and Seq.ID:6.

3. Claims: 7, 8, 17, 18, 26 all totally; 9-14, 21-24, 28-42 all partially.

   Idem as invention 1 but concerning Seq.ID:2 and Seq.ID:5.

# INTERNATIONAL SEARCH REPORT

...ormation on patent family members

Interr ...nal Application No
PCT/IB 98/00962

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 9608582 A | 21-03-1996 | AU 3468195 A | 29-03-1996 |
| | | BR 9508918 A | 21-10-1997 |
| | | CA 2199144 A | 21-03-1996 |
| | | EP 0804616 A | 05-11-1997 |
| | | JP 10504973 T | 19-05-1998 |
| | | NO 971111 A | 09-05-1997 |
| | | NZ 292494 A | 25-03-1998 |

Figure 1A

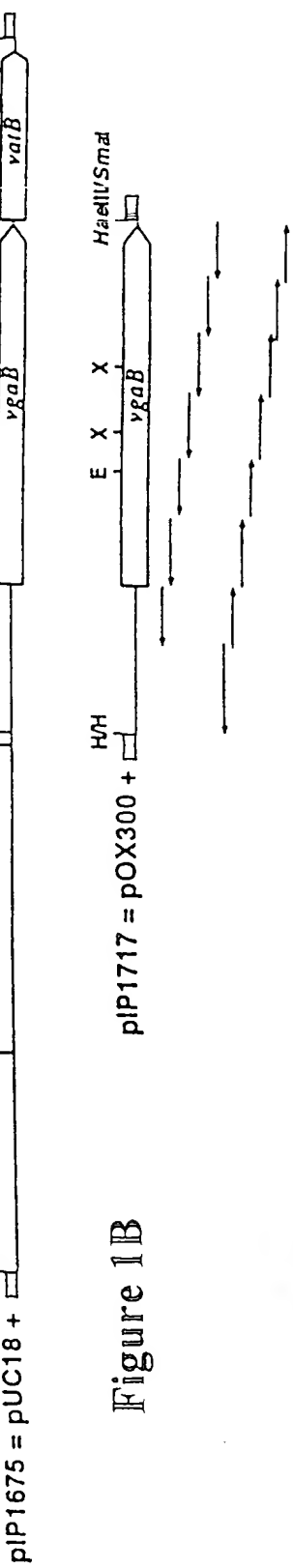



Figure 1B

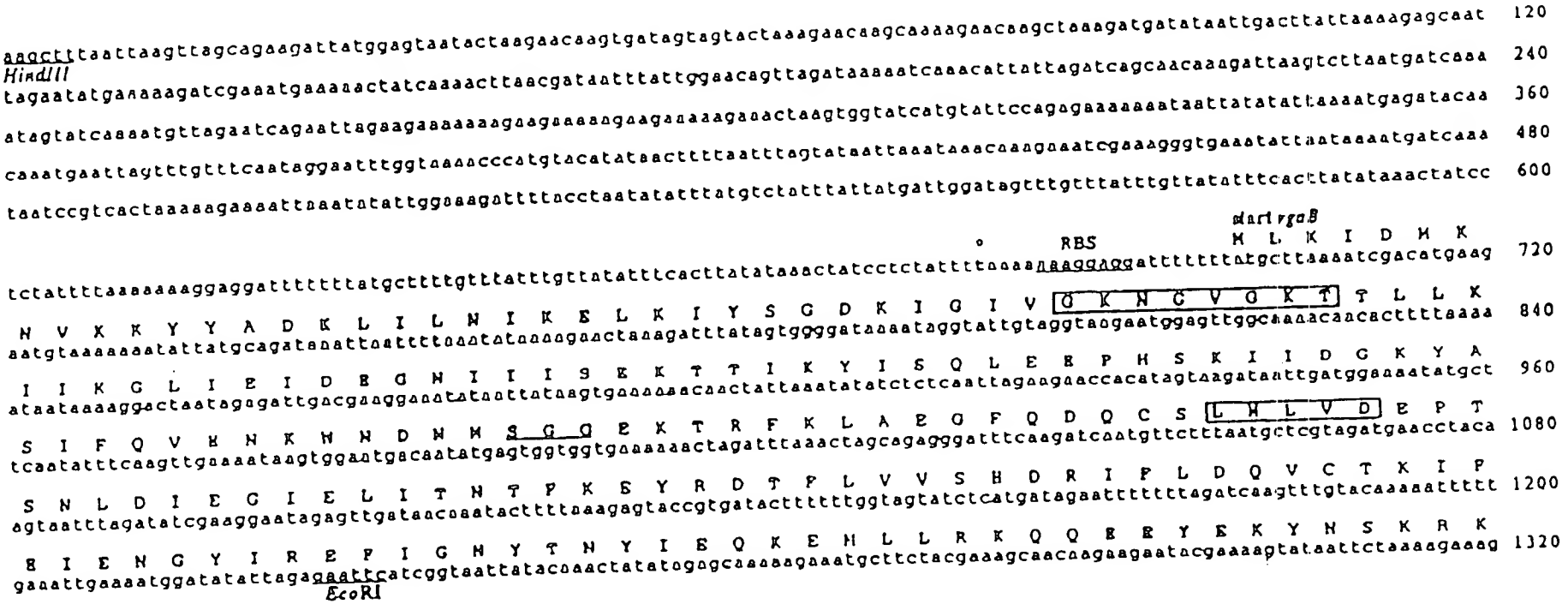



Figure 2

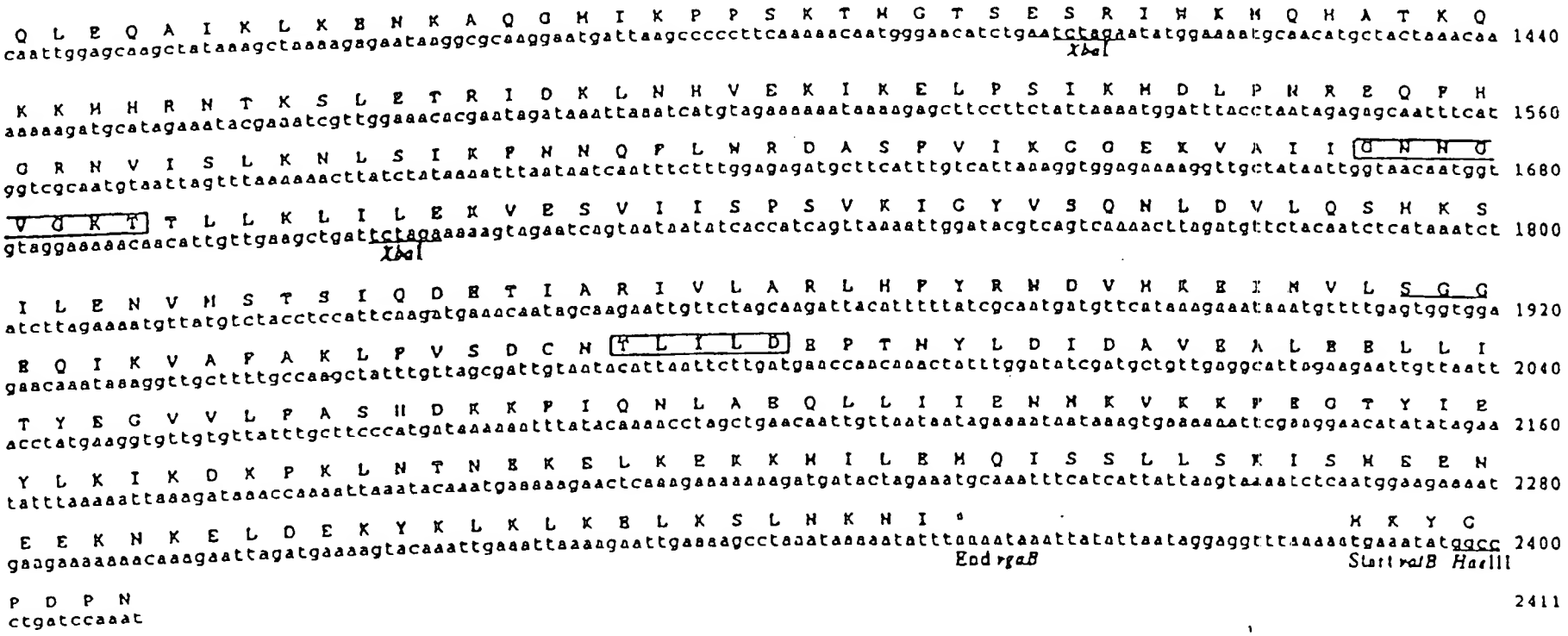



Figure 2 (cont.)

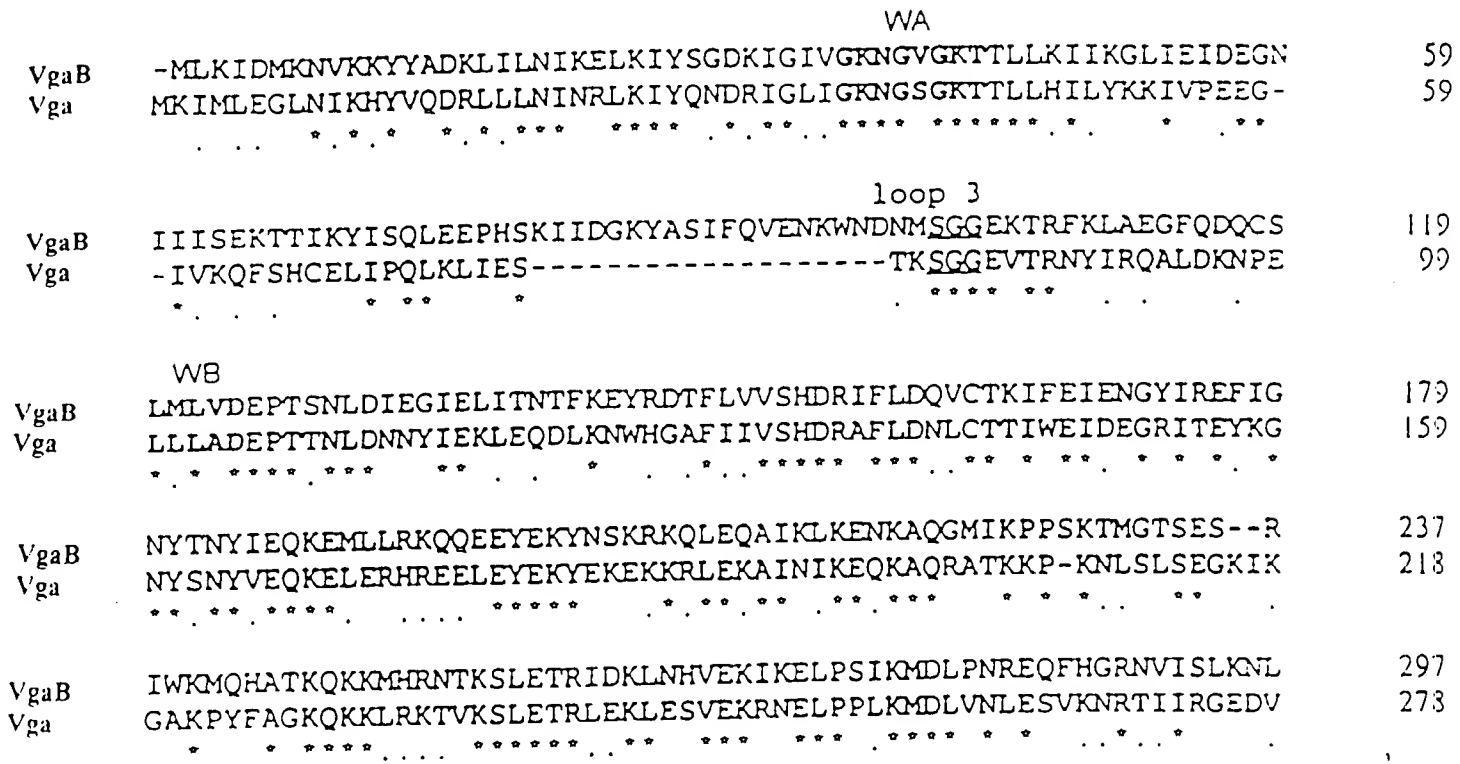

WA
VgaB -MLKIDMKNVKKYYADKLILNIKELKIYSGDKIGIVGKNGVGKTTLLKIIKGLIEIDEGN 59
Vga MKIMLEGLNIKHYVQDRLLLNINRLKIYQNDRIGLIGKNGSGKTTLLHILYKKIVPEEG- 59
loop 3
VgaB IIISEKTTIKYISQLEEPHSKIIDGKYASIFQVENKWNDNMSGGEKTRFKLAEGFQDQCS 119
Vga -IVKQFSHCELIPQLKLIES----------TKSGGEVTRNYIRQALDKNPE 99
WB
VgaB LMLVDEPTSNLDIEGIELITNTFKEYRDTFLVVSHDRIFLDQVCTKIFEIENGYIREFIG 179
Vga LLLADEPTTNLDNNYIEKLEQDLKNWHGAFIIVSHDRAFLDNLCTTIWEIDEGRITEYKG 159
VgaB NYTNYIEQKEMLLRKQQEEYEKYNSKRKQLEQAIKLKENKAQGMIKPPSKTMGTSES--R 237
Vga NYSNYVEQKELERHREELEYEKYEKEKKRLEKAINIKEQKAQRATKKP-KNLSLSEGKIK 218
VgaB IWKMQHATKQKKMHRNTKSLETRIDKLNHVEKIKELPSIKMDLPNREQFHGRNVISLKNL 297
Vga GAKPYFAGKQKKLRKTVKSLETRLEKLESVEKRNELPPLKMDLVNLESVKNRTIIRGEDV 278

Figure 3

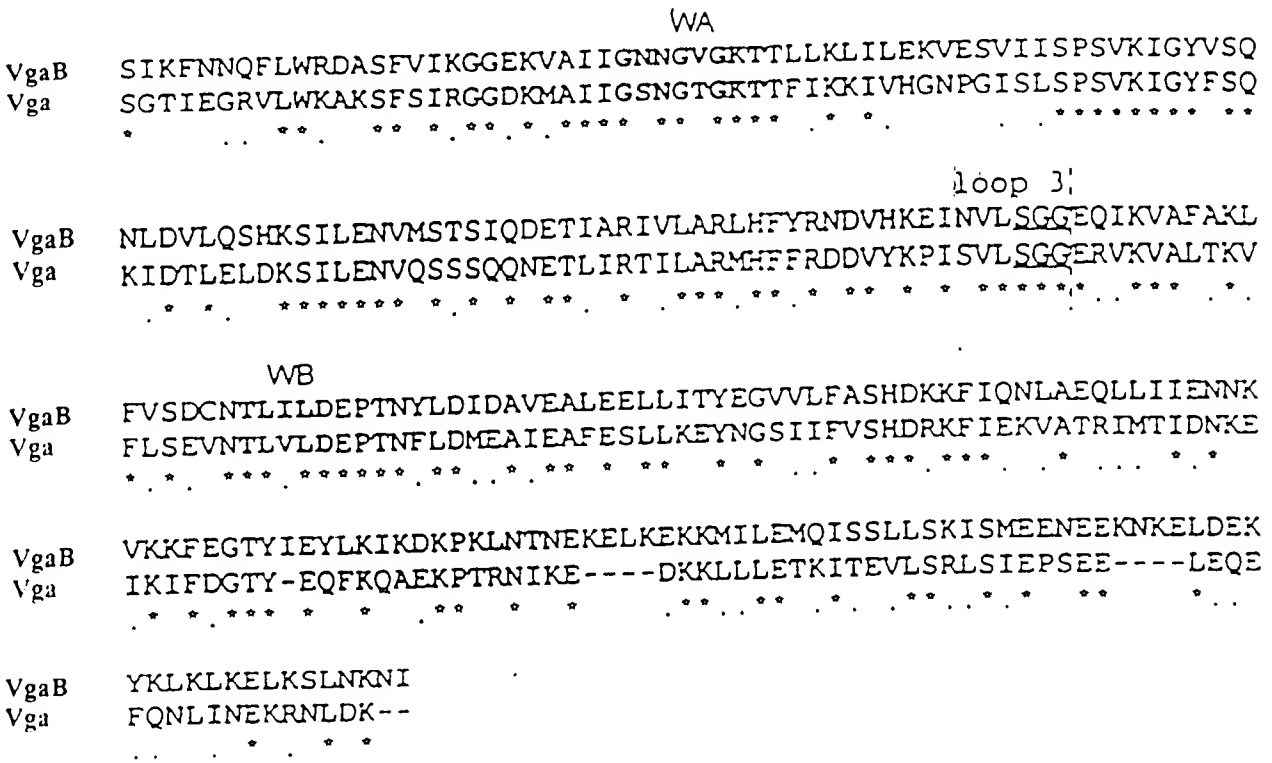



Figure 3 (cont.)

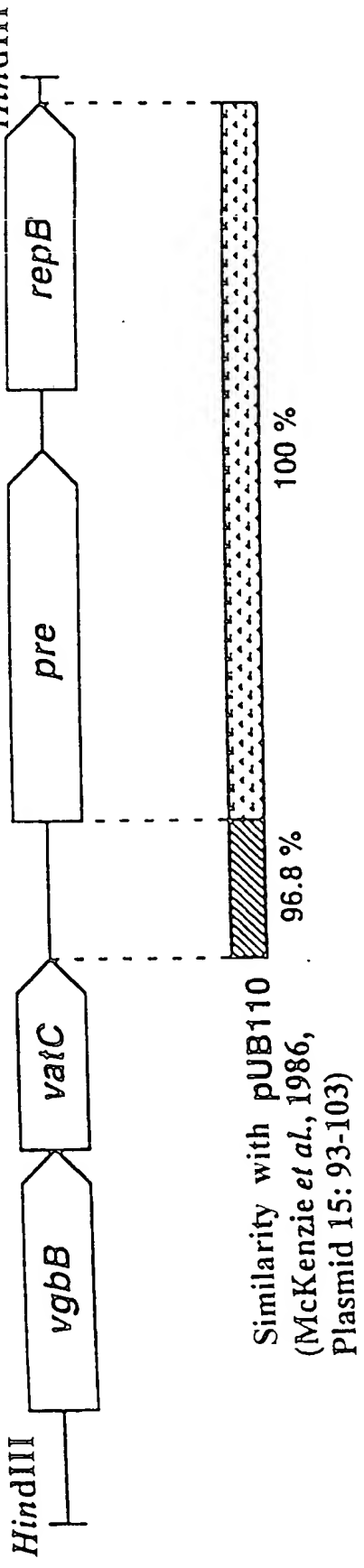
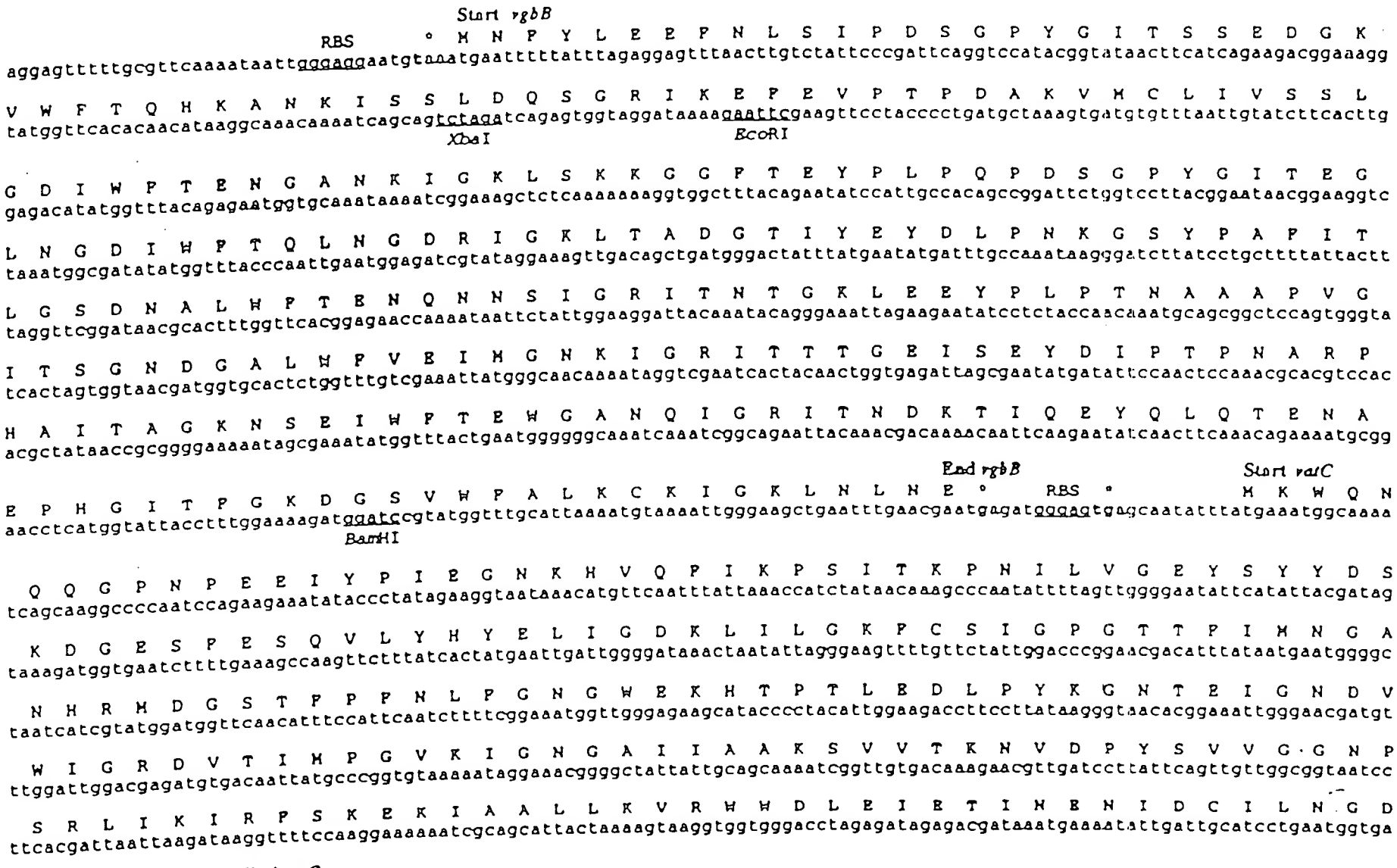

HindIII
repB
pre
vatC
vgbB
HindIII
100 %
96.8 %
Similarity with pUB110
(McKenzie et al., 1986,
Plasmid 15: 93-103)
1 kb

Figure 4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V W F T Q H K A N K I S S L D Q S G R I K E P E V P T P D A K V M C L I V S S L
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XbaI
EcoRI
G D I W P T E N G A N K I G K L S K K G G P T E Y P L P Q P D S G P Y G I T E G
gagacatatggtttacagagaatggtgcaaataaaatcggaaagctctcaaaaaaaggtggctttacagaatatccattgccacagccggattctggtccttacggaataacggaaggtc
L N G D I W P T Q L N G D R I G K L T A D G T I Y E Y D L P N K G S Y P A P I T
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L G S D N A L W P T E N Q N N S I G R I T N T G K L E E Y P L P T N A A A P V G
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I T S G N D G A L W P V E I M G N K I G R I T T T G E I S E Y D I P T P N A R P
tcactagtggtaacgatggtgcactctggtttgtcgaaattatgggcaacaaaataggtcgaatcactacaactggtgagattagcgaatatgatattccaactccaaacgcacgtccac
H A I T A G K N S E I W P T E W G A N Q I G R I T N D K T I Q E Y Q L Q T E N A
acgctataaccgcggggaaaaatagcgaaatatggtttactgaatgggggcaaatcaaatcggcagaattacaaacgacaaaacaattcaagaatatcaacttcaaacagaaaatgcgg
End rgbB
Start vacC
E P H G I T P G K D G S V W P A L K C K I G K L N L N E
RBS
M K W Q N
aacctcatggtattacctttggaaaagatggatccgtatggtttgcattaaaatgtaaaattgggaagctgaatttgaacgaatgagatgggagtgagcaatatttatgaaatggcaaaa
BamHI
Q Q G P N P E E I Y P I E G N K H V Q P I K P S I T K P N I L V G E Y S Y Y D S
tcagcaaggccccaatccagaagaaatataccctatagaaggtaataaacatgttcaatttattaaaccatctataacaaagcccaatattttagttggggaatattcatattacgatag
K D G E S P E S Q V L Y H Y E L I G D K L I L G K P C S I G P G T T P I M N G A
taaagatggtgaatcttttgaaagccaagttctttatcactatgaattgattggggataaactaatattagggaagttttgttctattggacccggaacgacatttataatgaatggggc
N H R M D G S T P P P N L P G N G W E K H T P T L E D L P Y K G N T E I G N D V
taatcatcgtatggatggttcaacatttccattcaatctttcggaaatggttgggagaagcataccctacattggaagaccttccttataagggtaacacggaaattgggaacgatgt
W I G R D V T I M P G V K I G N G A I I A A K S V V T K N V D P Y S V V G G N P
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S R L I K I R P S K E K I A A L L K V R W W D L E I E T I M E N I D C I L N G D
ttcacgattaattaagataaggttttccaaggaaaaaatcgcagcattactaaaagtaaggtggtgggacctagagatagagacgataaatgaaaatattgattgcatcctgaatggtga
End vacC
I K K V K R S
tataaaaaaggttaaaagaagttagaaaacgaattttgtttaggtta

Figure 5

PCR Primers

*vatC*

Oligo III 5'- atgaattcgcaaatcagcaagg-3'
*EcoR*I

Oligo IV 5'- tcgtctcgagctctaggtcc-3'
*Sac*I

Figure 6A

*vgbB*

Oligo V 5'- cagcagtctagatcagagtgg-3'
*Xba*I

Oligo VI 5'- catacggatccacctttlcc-3'
*Bam*H1

Figure 6B

**vga B**

Oligo I 5'-AAGTCGACTGACAATATGAGTGGTGG- 3'

Oligo II 5'-CTGCAGATGCCTCAACAGCATCGATATCC- 3'

Figure 6C

SEQ ID NO: 1

Seq *vgaB*

ATGCTTAAAATCGACATGAAGAATGTAAAAAAATATTATGCAGATAAATTAATTTTAAATATAAA
AAAGATTTATAGTGGGGATAAAATAGGTATTGTAGGTAAGAATGGAGTTGGCAAAACAACACTTT
TAATAAAAGGACTAATAGAGATTGACGAAGGAAATATAATTATAAGTGAAAAAACAACTATTAAA
TCTCAATTAGAAGAACCACATAGTAAGATAATTGATGGAAAATATGCTTCAATATTTCAAGTTGA
GTGGAATGACAATATGAGTGGTGGTGAAAAAACTAGATTTAAACTAGCAGAGGGATTTCAAGATC
CTTTAATGCTCGTAGATGAACCTACAAGTAATTTAGATATCGAAGGAATAGAGTTGATAACAAAT
AAAGAGTACCGTGATACTTTTTTGGTAGTATCTCATGATAGAATTTTTTTAGATCAAGTTTGTAC
TTTTGAAATTGAAAATGGATATATTAGAGAATTCATCGGTAATTATACAAACTATATAGAGCAAA
TGCTTCTACGAAAGCAACAAGAAGAATACGAAAAGTATAATTCTAAAAGAAAGCAATTGGAGCAA
AAGCTAAAAGAGAATAAGGCGCAAGGAATGATTAAGCCCCCTTCAAAAACAATGGGAACATCTGA
AATATGGAAAATGCAACATGCTACTAAACAAAAAAAGATGCATAGAAATACGAAATCGTTGGAAA
TAGATAAATTAAATCATGTAGAAAAAATAAAAGAGCTTCCTTCTATTAAAATGGATTTACCTAAT
CAATTTCATGGTCGCAATGTAATTAGTTTAAAAAACTTATCTATAAAATTTAATAATCAATTTCT
AGATGCTTCATTTGTCATTAAAGGTGGAGAAAAGGTTGCTATAATTGGTAACAATGGTGTAGGAA
CATTGTTGAAGCTGATTCTAGAAAAAGTAGAATCAGTAATAATATCACCATCAGTTAAAATTGGA
AGTCAAAACTTAGATGTTCTACAATCTCATAAATCTATCTTAGAAAATGTTATGTCTACCTCCAT
TGAAACAATAGCAAGAATTGTTCTAGCAAGATTACATTTTTATCGCAATGATGTTCATAAAGAAA
TTTTGAGTGGTGGAGAACAAATAAAGGTTGCTTTTGCCAAGCTATTTGTTAGCGATTGTAATACA
CTTGATGAACCAACAAACTATTTGGATATCGATGCTGTTGAGGCATTAGAAGAATTGTTAATTAC
AGGTGTTGTGTTATTTGCTTCCCATGATAAAAAATTTATACAAAACCTAGCTGAACAATTGTTAA
AAAATAATAAAGTGAAAAAATTCGAAGGAACATATATAGAATATTTAAAAATTAAAGATAAACCA
AATACAAATGAAAAAGAACTCAAAGAAAAAAAGATGATACTAGAAATGCAAATTTCATCATTATT
AATCTCAATGGAAGAAAATGAAGAAAAAAACAAAGAATTAGATGAAAAGTACAAATTGAAATTAA
TGAAAAGCCTAAATAAAAATATT

SEQ ID NO: 3

Seq *vgbB*

ATGAATTTTTATTTAGAGGAGTTTAACTTGTCTATTCCCGATTCAGGTCCATACGGTATAACTTC
AGACGGAAAGGTATGGTTCACACAACATAAGGCAAACAAAATCAGCAGTCTAGATCAGAGTGGTA
AAGAATTCGAAGTTCCTACCCCTGATGCTAAAGTGATGTGTTTAATTGTATCTTCACTTGGAGAC
TTTACAGAGAATGGTGCAAATAAAATCGGAAAGCTCTCAAAAAAAGGTGGCTTTACAGAATATCC
ACAGCCGGATTCTGGTCCTTACGGAATAACGGAAGGTCTAAATGGCGATATATGGTTTACCCAAT
GAGATCGTATAGGAAAGTTGACAGCTGATGGGACTATTTATGAATATGATTTGCCAAATAAGGGA
CCTGCTTTTATTACTTTAGGTTCGGATAACGCACTTTGGTTCACGGAGAACCAAAATAATTCTAT
GATTACAAATACAGGGAAATTAGAAGAATATCCTCTACCAACAAATGCAGCGGCTCCAGTGGGTA
GTGGTAACGATGGTGCACTCTGGTTTGTCGAAATTATGGGCAACAAAATAGGTCGAATCACTACA
GAGATTAGCGAATATGATATTCCAACTCCAAACGCACGTCCACACGCTATAACCGCGGGGAAAAA
AATATGGTTTACTGAATGGGGGGCAAATCAAATCGGCAGAATTACAAACGACAAAACAATTCAAG
AACTTCAAACAGAAAATGCGGAACCTCATGGTATTACCTTTGGAAAAGATGGATCCGTATGGTTT
AAATGTAAAATTGGGAAGCTGAATTTGAACGAA

Figure 7

SEQ ID NO: 2

Seq vatC

ATGAAATGGCAAAATCAGCAAGGCCCCAATCCAGAAGAAATATACCCTATAGAAGGTAATAAAC
AATTTATTAAACCATCTATAACAAAGCCCAATATTTTAGTTGGGGAATATTCATATTACGATAG
TGGTGAATCTTTTGAAAGCCAAGTTCTTTATCACTATGAATTGATTGGGGATAAACTAATATTA
TTTTGTTCTATTGGACCCGGAACGACATTTATAATGAATGGGGCTAATCATCGTATGGATGGTT
TTCCATTCAATCTTTTCGGAAATGGTTGGGAGAAGCATACCCCTACATTGGAAGACCTTCCTTA
TAACACGGAAATTGGGAACGATGTTTGGATTGGACGAGATGTGACAATTATGCCCGGTGTAAAA
AACGGGGCTATTATTGCAGCAAAATCGGTTGTGACAAAGAACGTTGATCCTTATTCAGTTGTTG
ATCCTTCACGATTAATTAAGATAAGGTTTTCCAAGGAAAAAATCGCAGCATTACTAAAAGTAAG
GGACCTAGAGATAGAGACGATAAATGAAAATATTGATTGCATCCTGAATGGTGATATAAAAAAG
AGAAGT

Figure 7 (cont.)

SEQ ID NO: 4

VgaB

MLKIDMKNVKKYYADKLILNIKELKIYSGDKIGIVGKNGVGKTTLLKIIK
GLIEIDEGNIIISEKTTIKYISQLEEPHSKIIDGKYASIFQVENKWNDNM
SGGEKTRFKLAEGFQDQCSLMLVDEPTSNLDIEGIELITNTFKEYRDTFL
VVSHDRIFLDQVCTKIFEIENGYIREFIGNYTNYIEQKEMLLRKQQEEYE
KYNSKRKQLEQAIKLKENKAQGMIKPPSKTMGTSESRIWKMQHATKQKKM
HRNTKSLETRIDKLNHVEKIKELPSIKMDLPNREQFHGRNVISLKNLSIK
FNNQFLWRDASFVIKGGEKVAIIGNNGVGKTTLLKLILEKVESVIISPSV
KIGYVSQNLDVLQSHKSILENVMSTSIQDETIARIVLARLHFYRNDVHKE
INVLSGGEQIKVAFAKLFVSDCNTLILDEPTNYLDIDAVEALEELLITYE
GVVLFASHDKKFIQNLAEQLLIIENNKVKKFEGTYIEYLKIKDKPKLNTN
EKELKEKKMILEMQISSLLSKISMEENEEKNKELDEKYKLKLKELKSLNK
NI

SEQ ID NO: 6

VgbB

MNFYLEEFNLSIPDSGPYGITSSEDGKVWFTQHKANKISSLDQSGRIKEF
EVPTPDAKVMCLIVSSLGDIWFTENGANKIGKLSKKGGFTEYPLPQPDSG
PGITEGLNGDIWFTQLNGDRIGKLTADGTIYEYDLPNKGSYPAFITLGSD
NALWFTENQNNSIGRITNTGKLEEYPLPTNAAAPVGITSGNDGALWFVEI
MGNKIGRITTTGEISEYDIPTPNARPHAITAGKNSEIWFTEWGANQIGRI
TNDKTIQEYQLQTENAEPHGITFGKDGSVWFALKCKIGKLNLNE

SEQ ID NO: 5

VatC

MKWQNQQGPNPEEIYPIEGNKHVQFIKPSITKPNILVGEYSYYDSKDGES
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NGWEKHTPTLEDLPYKGNTEIGNDVWIGRDVTIMPGVKIGNGAIIAAKSV
VTKNVDPYSVVGGNPSRLIKIRFSKEKIAALLKVRWWDLEIETINENIDC
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Figure 7 (cont.)

SEQ ID NO: 7 K S I L E N V VgaB

SEQ ID NO: 11 1795 5′– aaa tct atc tta gaa aat gtt –3′ 1815 *vgaB*
g agc t

SEQ ID NO: 8 N Y T N Y I E Q K E VgaB
S V

SEQ ID NO: 12 1237 5′– aat tat aca aac tat ata gag caa aaa gaa –3′ 1266 *vgaB*
gt gtt a

SEQ ID NO: 9 I M N G A N H R M VatC

SEQ ID NO: 13 1187 5′– ata atg aat ggg gct aat cat cgt atg –3′ 1213 *vatC*
t a c a a g t

SEQ ID NO: 10 G N D V W I G VatC

SEQ ID NO: 14 1310 5′– ggg aac gat gtt tgg att gga –3′ 1330 *vatC*
a t g t a t a a t

Figure 7 (cont.)

LISTE DE SÉQUENCES

<110> INSTITUT PASTEUR

<120> POLYNUCLEOTIDES AND THEIR USE FOR DETECTING RESISTANCE TO STREPTOGRAMIN A OR TO STREPTOGRAMIN B AND RELETED COMPOUNDS.

<130> D17589

<150> US 60/050 380
<151> 1997-06-20

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ataaatgttt tgagtggtgg agaacaaata aaggttgctt ttgccaagct atttgttagc 1260
gattgtaata cattaattct tgatgaacca acaaactatt tggatatcga tgctgttgag 1320
gcattagaag aattgttaat tacctatgaa ggtgttgtgt tatttgcttc ccatgataaa 1380
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ttcgaaggaa catatataga atatttaaaa attaaagata aaccaaaatt aaatacaaat 1500
gaaaaagaac tcaaagaaaa aaagatgata ctagaaatgc aaatttcatc attattaagt 1560
aaaatctcaa tggaagaaaa tgaagaaaaa aacaaagaat tagatgaaaa gtacaaattg 1620
aaattaaaag aattgaaaag cctaaataaa aatatt 1656

<210> 2
<211> 636
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<213> Staphyloccocus

<220>
<223> vatC

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tcatattacg atagtaaaga tggtgaatct tttgaaagcc aagttcttta tcactatgaa 180
ttgattgggg ataaactaat attagggaag ttttgttcta ttggacccgg aacgacattt 240
ataatgaatg gggctaatca tcgtatggat ggttcaacat ttccattcaa tcttttcgga 300
aatggttggg agaagcatac ccctacattg gaagaccttc cttataaggg taacacggaa 360
attgggaacg atgtttggat tggacgagat gtgacaatta tgcccggtgt aaaaatagga 420
aacggggcta ttattgcagc aaaatcggtt gtgacaaaga acgttgatcc ttattcagtt 480
gttggcggta atccttcacg attaattaag ataaggtttt ccaaggaaaa aatcgcagca 540
ttactaaaag taaggtggtg ggacctagag atagagacga taaatgaaaa tattgattgc 600
atcctgaatg gtgatataaa aaaggttaaa agaagt 636

<210> 3
<211> 885
<212> ADN
<213> Staphyloccocus

<220>
<223> vgbB

<400> 3
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ctagatcaga gtggtaggat aaaagaattc gaagttccta cccctgatgc taaagtgatg 180
tgtttaattg tatcttcact tggagacata tggtttacag agaatggtgc aaataaaatc 240
ggaaagctct caaaaaaagg tggctttaca gaatatccat tgccacagcc ggattctggt 300
ccttacggaa taacggaagg tctaaatggc gatatatggt ttacccaatt gaatggagat 360
cgtataggaa agttgacagc tgatgggact atttatgaat atgatttgcc aaataaggga 420
tcttatcctg cttttattac tttaggttcg gataacgcac tttggttcac ggagaaccaa 480
aataattcta ttggaaggat tacaaataca gggaaattag aagaatatcc tctaccaaca 540
aatgcagcgg ctccagtggg tatcactagt ggtaacgatg gtgcactctg gtttgtcgaa 600
attatgggca acaaaatagg tcgaatcact acaactggtg agattagcga atatgatatt 660
ccaactccaa acgcacgtcc acacgctata accgcgggga aaaatagcga aatatggttt 720
actgaatggg gggcaaatca aatcggcaga attacaaacg acaaaacaat tcaagaatat 780
caacttcaaa cagaaaatgc ggaacctcat ggtattacct ttggaaaaga tggatccgta 840
tggtttgcat taaaatgtaa aattgggaag ctgaatttga acgaa 885

<210> 4
<211> 552
<212> PRT
<213> Staphyloccocus

<220>
<223> vgaB

<400> 4
Met Leu Lys Ile Asp Met Lys Asn Val Lys Lys Tyr Tyr Ala Asp Lys
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Leu Ile Leu Asn Ile Lys Glu Leu Lys Ile Tyr Ser Gly Asp Lys Ile
            20                  25                  30

Gly Ile Val Gly Lys Asn Gly Val Gly Lys Thr Thr Leu Leu Lys Ile
        35                  40                  45

```
Ile Lys Gly Leu Ile Glu Ile Asp Glu Gly Asn Ile Ile Ile Ser Glu
     50                  55                  60

Lys Thr Thr Ile Lys Tyr Ile Ser Gln Leu Glu Glu Pro His Ser Lys
 65                  70                  75                  80

Ile Ile Asp Gly Lys Tyr Ala Ser Ile Phe Gln Val Glu Asn Lys Trp
                 85                  90                  95

Asn Asp Asn Met Ser Gly Gly Glu Lys Thr Arg Phe Lys Leu Ala Glu
            100                 105                 110

Gly Phe Gln Asp Gln Cys Ser Leu Met Leu Val Asp Glu Pro Thr Ser
        115                 120                 125

Asn Leu Asp Ile Glu Gly Ile Glu Leu Ile Thr Asn Thr Phe Lys Glu
    130                 135                 140

Tyr Arg Asp Thr Phe Leu Val Val Ser His Asp Arg Ile Phe Leu Asp
145                 150                 155                 160

Gln Val Cys Thr Lys Ile Phe Glu Ile Glu Asn Gly Tyr Ile Arg Glu
                165                 170                 175

Phe Ile Gly Asn Tyr Thr Asn Tyr Ile Glu Gln Lys Glu Met Leu Leu
            180                 185                 190

Arg Lys Gln Gln Glu Glu Tyr Glu Lys Tyr Asn Ser Lys Arg Lys Gln
        195                 200                 205

Leu Glu Gln Ala Ile Lys Leu Lys Glu Asn Lys Ala Gln Gly Met Ile
    210                 215                 220

Lys Pro Pro Ser Lys Thr Met Gly Thr Ser Glu Ser Arg Ile Trp Lys
225                 230                 235                 240

Met Gln His Ala Thr Lys Gln Lys Lys Met His Arg Asn Thr Lys Ser
                245                 250                 255

Leu Glu Thr Arg Ile Asp Lys Leu Asn His Val Glu Lys Ile Lys Glu
            260                 265                 270

Leu Pro Ser Ile Lys Met Asp Leu Pro Asn Arg Glu Gln Phe His Gly
        275                 280                 285

Arg Asn Val Ile Ser Leu Lys Asn Leu Ser Ile Lys Phe Asn Asn Gln
    290                 295                 300

Phe Leu Trp Arg Asp Ala Ser Phe Val Ile Lys Gly Gly Glu Lys Val
305                 310                 315                 320

Ala Ile Ile Gly Asn Asn Gly Val Gly Lys Thr Thr Leu Leu Lys Leu
                325                 330                 335

Ile Leu Glu Lys Val Glu Ser Val Ile Ile Ser Pro Ser Val Lys Ile
            340                 345                 350

Gly Tyr Val Ser Gln Asn Leu Asp Val Leu Gln Ser His Lys Ser Ile
        355                 360                 365
```

```
Leu Glu Asn Val Met Ser Thr Ser Ile Gln Asp Glu Thr Ile Ala Arg
    370                 375                 380

Ile Val Leu Ala Arg Leu His Phe Tyr Arg Asn Asp Val His Lys Glu
385                 390                 395                 400

Ile Asn Val Leu Ser Gly Gly Glu Gln Ile Lys Val Ala Phe Ala Lys
                405                 410                 415

Leu Phe Val Ser Asp Cys Asn Thr Leu Ile Leu Asp Glu Pro Thr Asn
            420                 425                 430

Tyr Leu Asp Ile Asp Ala Val Glu Ala Leu Glu Glu Leu Leu Ile Thr
        435                 440                 445

Tyr Glu Gly Val Val Leu Phe Ala Ser His Asp Lys Lys Phe Ile Gln
    450                 455                 460

Asn Leu Ala Glu Gln Leu Leu Ile Ile Glu Asn Asn Lys Val Lys Lys
465                 470                 475                 480

Phe Glu Gly Thr Tyr Ile Glu Tyr Leu Lys Ile Lys Asp Lys Pro Lys
                485                 490                 495

Leu Asn Thr Asn Glu Lys Glu Leu Lys Glu Lys Lys Met Ile Leu Glu
            500                 505                 510

Met Gln Ile Ser Ser Leu Leu Ser Lys Ile Ser Met Glu Glu Asn Glu
        515                 520                 525

Glu Lys Asn Lys Glu Leu Asp Glu Lys Tyr Lys Leu Lys Leu Lys Glu
    530                 535                 540

Leu Lys Ser Leu Asn Lys Asn Ile
545                 550
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<210> 5
<211> 212
<212> PRT
<213> Staphyloccocus

<220>
<223> vatC

<400> 5

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Met Lys Trp Gln Asn Gln Gln Gly Pro Asn Pro Glu Glu Ile Tyr Pro
  1               5                  10                  15

Ile Glu Gly Asn Lys His Val Gln Phe Ile Lys Pro Ser Ile Thr Lys
            20                  25                  30

Pro Asn Ile Leu Val Gly Glu Tyr Ser Tyr Tyr Asp Ser Lys Asp Gly
        35                  40                  45

Glu Ser Phe Glu Ser Gln Val Leu Tyr His Tyr Glu Leu Ile Gly Asp
    50                  55                  60

Lys Leu Ile Leu Gly Lys Phe Cys Ser Ile Gly Pro Gly Thr Thr Phe
65                  70                  75                  80
```

```
Ile Met Asn Gly Ala Asn His Arg Met Asp Gly Ser Thr Phe Pro Phe
                 85                  90                  95

Asn Leu Phe Gly Asn Gly Trp Glu Lys His Thr Pro Thr Leu Glu Asp
            100                 105                 110

Leu Pro Tyr Lys Gly Asn Thr Glu Ile Gly Asn Asp Val Trp Ile Gly
        115                 120                 125

Arg Asp Val Thr Ile Met Pro Gly Val Lys Ile Gly Asn Gly Ala Ile
    130                 135                 140

Ile Ala Ala Lys Ser Val Val Thr Lys Asn Val Asp Pro Tyr Ser Val
145                 150                 155                 160

Val Gly Gly Asn Pro Ser Arg Leu Ile Lys Ile Arg Phe Ser Lys Glu
                165                 170                 175

Lys Ile Ala Ala Leu Leu Lys Val Arg Trp Trp Asp Leu Glu Ile Glu
            180                 185                 190

Thr Ile Asn Glu Asn Ile Asp Cys Ile Leu Asn Gly Asp Ile Lys Lys
        195                 200                 205

Val Lys Arg Ser
    210
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<210> 6
<211> 294
<212> PRT
<213> Staphyloccocus

<220>
<223> vgbB

<400> 6

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Met Asn Phe Tyr Leu Glu Glu Phe Asn Leu Ser Ile Pro Asp Ser Gly
  1               5                  10                  15

Pro Tyr Gly Ile Thr Ser Ser Glu Asp Gly Lys Val Trp Phe Thr Gln
             20                  25                  30

His Lys Ala Asn Lys Ile Ser Ser Leu Asp Gln Ser Gly Arg Ile Lys
         35                  40                  45

Glu Phe Glu Val Pro Thr Pro Asp Ala Lys Val Met Cys Leu Ile Val
     50                  55                  60

Ser Ser Leu Gly Asp Ile Trp Phe Thr Glu Asn Gly Ala Asn Lys Ile
 65                  70                  75                  80

Gly Lys Leu Ser Lys Lys Gly Gly Phe Thr Glu Tyr Pro Leu Pro Gln
                 85                  90                  95

Pro Asp Ser Gly Pro Gly Ile Thr Glu Gly Leu Asn Gly Asp Ile Trp
            100                 105                 110

Phe Thr Gln Leu Asn Gly Asp Arg Ile Gly Lys Leu Thr Ala Asp Gly
```

```
        115                 120                 125

Thr Ile Tyr Glu Tyr Asp Leu Pro Asn Lys Gly Ser Tyr Pro Ala Phe
    130                 135                 140

Ile Thr Leu Gly Ser Asp Asn Ala Leu Trp Phe Thr Glu Asn Gln Asn
145                 150                 155                 160

Asn Ser Ile Gly Arg Ile Thr Asn Thr Gly Lys Leu Glu Glu Tyr Pro
                165                 170                 175

Leu Pro Thr Asn Ala Ala Ala Pro Val Gly Ile Thr Ser Gly Asn Asp
            180                 185                 190

Gly Ala Leu Trp Phe Val Glu Ile Met Gly Asn Lys Ile Gly Arg Ile
        195                 200                 205

Thr Thr Thr Gly Glu Ile Ser Glu Tyr Asp Ile Pro Thr Pro Asn Ala
    210                 215                 220

Arg Pro His Ala Ile Thr Ala Gly Lys Asn Ser Glu Ile Trp Phe Thr
225                 230                 235                 240

Glu Trp Gly Ala Asn Gln Ile Gly Arg Ile Thr Asn Asp Lys Thr Ile
                245                 250                 255

Gln Glu Tyr Gln Leu Gln Thr Glu Asn Ala Glu Pro His Gly Ile Thr
            260                 265                 270

Phe Gly Lys Asp Gly Ser Val Trp Phe Ala Leu Lys Cys Lys Ile Gly
        275                 280                 285

Lys Leu Asn Leu Asn Glu
    290
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<210> 7
<211> 7
<212> PRT
<213> Staphyloccocus

<220>
<223> vgaB

<400> 7
Lys Ser Ile Leu Glu Asn Val
1               5

<210> 8
<211> 10
<212> PRT
<213> Staphyloccocus

<220>
<223> vgaB

<400> 8
Asn Tyr Thr Asn Tyr Ile Glu Gln Lys Glu

1 5 10

<210> 9
<211> 9
<212> PRT
<213> Staphyloccocus

<220>
<223> vatC

<400> 9
Ile Met Asn Gly Ala Asn His Arg Met
1 5

<210> 10
<211> 7
<212> PRT
<213> Staphyloccocus

<220>
<223> vatC

<400> 10
Gly Asn Asp Val Trp Ile Gly
1 5

<210> 11
<211> 21
<212> ADN
<213> Staphyloccocus

<220>
<223> vgaB

<400> 11
aaatctatct tagaaaatgt t 21

<210> 12
<211> 30
<212> ADN
<213> Staphyloccocus

<220>
<223> vgaB

<400> 12
aattatacaa actatataga gcaaaaagaa 30

<210> 13
<211> 27
<212> ADN
<213> Staphyloccocus

<220>
<223> vatC

<400> 13
ataatgaatg gggctaatca tcgtatg 27

<210> 14
<211> 21
<212> ADN
<213> Staphyloccocus

<220>
<223> vatC

<400> 14
gggaacgatg tttggattgg a 21

<210> 15
<211> 2411
<212> ADN
<213> Staphyloccocus

<220>
<221> CDS
<222> (700)..(2355)

<220>
<221> CDS
<222> (2388)..(2411)

<220>
<223> vgaB et vatB

<400> 15
aagctttaat taagttagca gaagattatg gagtaatact aagaacaagt gatagtagta 60

ctaaagaaca agcaaaagaa caagctaaag atgatataat tgacttatta aaagagcaat 120

tagaatatga aaaagatcga aatgaaaaac tatcaaaact taacgataat ttattggaac 180

agttagataa aaatcaaaca ttattagatc agcaacaaag attaagtctt aatgatcaaa 240

atagtatcaa aatgttagaa tcagaattag aagaaaaaaa gaagaaaaag aagaaaaaga 300

aactaagtgg tatcatgtat tccagagaaa aaaataatta tatattaaaa tgagatacaa 360

caaatgaatt agtttgtttc aataggaatt tggtaaaacc catgtacata taacttttaa 420

tttagtataa ttaaataaac aaagaaatcg aaagggtgaa atattaataa aatgatcaaa 480

taatccgtca ctaaaaagaa aattaaatat attggaaaga ttttacctaa tatatttatg 540

tctatttatt atgattggat agtttgttta tttgttatat ttcacttata taaactatcc 600

tctattttaa aaaaaggagg attttttat gcttttgttt atttgttata tttcacttat 660

ataaactatc ctctatttta aaaaaaggag gatttttttatg ctt aaa atc gac 714
Met Leu Lys Ile Asp
1 5

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atg aag aat gta aaa aaa tat tat gca gat aaa tta att tta aat ata   762
Met Lys Asn Val Lys Lys Tyr Tyr Ala Asp Lys Leu Ile Leu Asn Ile
                 10                  15                  20

aaa gaa cta aag att tat agt ggg gat aaa ata ggt att gta ggt aag   810
Lys Glu Leu Lys Ile Tyr Ser Gly Asp Lys Ile Gly Ile Val Gly Lys
             25                  30                  35

aat gga gtt ggc aaa aca aca ctt tta aaa ata ata aaa gga cta ata   858
Asn Gly Val Gly Lys Thr Thr Leu Leu Lys Ile Ile Lys Gly Leu Ile
         40                  45                  50

gag att gac gaa gga aat ata att ata agt gaa aaa aca act att aaa   906
Glu Ile Asp Glu Gly Asn Ile Ile Ile Ser Glu Lys Thr Thr Ile Lys
     55                  60                  65

tat atc tct caa tta gaa gaa cca cat agt aag ata att gat gga aaa   954
Tyr Ile Ser Gln Leu Glu Glu Pro His Ser Lys Ile Ile Asp Gly Lys
 70                  75                  80                  85

tat gct tca ata ttt caa gtt gaa aat aag tgg aat gac aat atg agt   1002
Tyr Ala Ser Ile Phe Gln Val Glu Asn Lys Trp Asn Asp Asn Met Ser
                 90                  95                 100

ggt ggt gaa aaa act aga ttt aaa cta gca gag gga ttt caa gat caa   1050
Gly Gly Glu Lys Thr Arg Phe Lys Leu Ala Glu Gly Phe Gln Asp Gln
             105                 110                 115

tgt tct tta atg ctc gta gat gaa cct aca agt aat tta gat atc gaa   1098
Cys Ser Leu Met Leu Val Asp Glu Pro Thr Ser Asn Leu Asp Ile Glu
         120                 125                 130

gga ata gag ttg ata aca aat act ttt aaa gag tac cgt gat act ttt   1146
Gly Ile Glu Leu Ile Thr Asn Thr Phe Lys Glu Tyr Arg Asp Thr Phe
     135                 140                 145

ttg gta gta tct cat gat aga att ttt tta gat caa gtt tgt aca aaa   1194
Leu Val Val Ser His Asp Arg Ile Phe Leu Asp Gln Val Cys Thr Lys
150                 155                 160                 165

att ttt gaa att gaa aat gga tat att aga gaa ttc atc ggt aat tat   1242
Ile Phe Glu Ile Glu Asn Gly Tyr Ile Arg Glu Phe Ile Gly Asn Tyr
                 170                 175                 180

aca aac tat ata gag caa aaa gaa atg ctt cta cga aag caa caa gaa   1290
Thr Asn Tyr Ile Glu Gln Lys Glu Met Leu Leu Arg Lys Gln Gln Glu
             185                 190                 195

gaa tac gaa aag tat aat tct aaa aga aag caa ttg gag caa gct ata   1338
Glu Tyr Glu Lys Tyr Asn Ser Lys Arg Lys Gln Leu Glu Gln Ala Ile
         200                 205                 210

aag cta aaa gag aat aag gcg caa gga atg att aag ccc cct tca aaa   1386
Lys Leu Lys Glu Asn Lys Ala Gln Gly Met Ile Lys Pro Pro Ser Lys
     215                 220                 225

aca atg gga aca tct gaa tct aga ata tgg aaa atg caa cat gct act   1434
Thr Met Gly Thr Ser Glu Ser Arg Ile Trp Lys Met Gln His Ala Thr
230                 235                 240                 245
```

```
aaa caa aaa aag atg cat aga aat acg aaa tcg ttg gaa aca cga ata   1482
Lys Gln Lys Lys Met His Arg Asn Thr Lys Ser Leu Glu Thr Arg Ile
                250                 255                 260

gat aaa tta aat cat gta gaa aaa ata aaa gag ctt cct tct att aaa   1530
Asp Lys Leu Asn His Val Glu Lys Ile Lys Glu Leu Pro Ser Ile Lys
            265                 270                 275

atg gat tta cct aat aga gag caa ttt cat ggt cgc aat gta att agt   1578
Met Asp Leu Pro Asn Arg Glu Gln Phe His Gly Arg Asn Val Ile Ser
        280                 285                 290

tta aaa aac tta tct ata aaa ttt aat aat caa ttt ctt tgg aga gat   1626
Leu Lys Asn Leu Ser Ile Lys Phe Asn Asn Gln Phe Leu Trp Arg Asp
    295                 300                 305

gct tca ttt gtc att aaa ggt gga gaa aag gtt gct ata att ggt aac   1674
Ala Ser Phe Val Ile Lys Gly Gly Glu Lys Val Ala Ile Ile Gly Asn
310                 315                 320                 325

aat ggt gta gga aaa aca aca ttg ttg aag ctg att cta gaa aaa gta   1722
Asn Gly Val Gly Lys Thr Thr Leu Leu Lys Leu Ile Leu Glu Lys Val
                330                 335                 340

gaa tca gta ata ata tca cca tca gtt aaa att gga tac gtc agt caa   1770
Glu Ser Val Ile Ile Ser Pro Ser Val Lys Ile Gly Tyr Val Ser Gln
            345                 350                 355

aac tta gat gtt cta caa tct cat aaa tct atc tta gaa aat gtt atg   1818
Asn Leu Asp Val Leu Gln Ser His Lys Ser Ile Leu Glu Asn Val Met
        360                 365                 370

tct acc tcc att caa gat gaa aca ata gca aga att gtt cta gca aga   1866
Ser Thr Ser Ile Gln Asp Glu Thr Ile Ala Arg Ile Val Leu Ala Arg
    375                 380                 385

tta cat ttt tat cgc aat gat gtt cat aaa gaa ata aat gtt ttg agt   1914
Leu His Phe Tyr Arg Asn Asp Val His Lys Glu Ile Asn Val Leu Ser
390                 395                 400                 405

ggt gga gaa caa ata aag gtt gct ttt gcc aag cta ttt gtt agc gat   1962
Gly Gly Glu Gln Ile Lys Val Ala Phe Ala Lys Leu Phe Val Ser Asp
                410                 415                 420

tgt aat aca tta att ctt gat gaa cca aca aac tat ttg gat atc gat   2010
Cys Asn Thr Leu Ile Leu Asp Glu Pro Thr Asn Tyr Leu Asp Ile Asp
            425                 430                 435

gct gtt gag gca tta gaa gaa ttg tta att acc tat gaa ggt gtt gtg   2058
Ala Val Glu Ala Leu Glu Glu Leu Leu Ile Thr Tyr Glu Gly Val Val
        440                 445                 450

tta ttt gct tcc cat gat aaa aaa ttt ata caa aac cta gct gaa caa   2106
Leu Phe Ala Ser His Asp Lys Lys Phe Ile Gln Asn Leu Ala Glu Gln
    455                 460                 465

ttg tta ata ata gaa aat aat aaa gtg aaa aaa ttc gaa gga aca tat   2154
Leu Leu Ile Ile Glu Asn Asn Lys Val Lys Lys Phe Glu Gly Thr Tyr
470                 475                 480                 485

ata gaa tat tta aaa att aaa gat aaa cca aaa tta aat aca aat gaa   2202
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```
Ile Glu Tyr Leu Lys Ile Lys Asp Lys Pro Lys Leu Asn Thr Asn Glu
                490                 495                 500

aaa gaa ctc aaa gaa aaa aag atg ata cta gaa atg caa att tca tca   2250
Lys Glu Leu Lys Glu Lys Lys Met Ile Leu Glu Met Gln Ile Ser Ser
            505                 510                 515

tta tta agt aaa atc tca atg gaa gaa aat gaa gaa aaa aac aaa gaa   2298
Leu Leu Ser Lys Ile Ser Met Glu Glu Asn Glu Glu Lys Asn Lys Glu
        520                 525                 530

tta gat gaa aag tac aaa ttg aaa tta aaa gaa ttg aaa agc cta aat   2346
Leu Asp Glu Lys Tyr Lys Leu Lys Leu Lys Glu Leu Lys Ser Leu Asn
    535                 540                 545

aaa aat att taaaataaat tatattaata ggaggtttaa aa atg aaa tat ggc   2399
Lys Asn Ile                                 Met Lys Tyr Gly
550                                                 555

cct gat cca aat                                                   2411
Pro Asp Pro Asn
            560
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<210> 16
<211> 1607
<212> ADN
<213> Staphyloccocus

<220>
<221> CDS
<222> (39)..(926)

<220>
<221> CDS
<222> (947)..(1585)

<220>
<223> vgbB et vatC

<400> 16

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aggagttttt gcgttcaaaa taattgggag gaatgtaa atg aat ttt tat tta gag 56
                                          Met Asn Phe Tyr Leu Glu
                                            1               5

gag ttt aac ttg tct att ccc gat tca ggt cca tac ggt ata act tca   104
Glu Phe Asn Leu Ser Ile Pro Asp Ser Gly Pro Tyr Gly Ile Thr Ser
             10                  15                  20

tca gaa gac gga aag gta tgg ttc aca caa cat aag gca aac aaa atc   152
Ser Glu Asp Gly Lys Val Trp Phe Thr Gln His Lys Ala Asn Lys Ile
         25                  30                  35

agc agt cta gat cag agt ggt agg ata aaa gaa ttc gaa gtt cct acc   200
Ser Ser Leu Asp Gln Ser Gly Arg Ile Lys Glu Phe Glu Val Pro Thr
     40                  45                  50

cct gat gct aaa gtg atg tgt tta att gta tct tca ctt gga gac ata   248
Pro Asp Ala Lys Val Met Cys Leu Ile Val Ser Ser Leu Gly Asp Ile
 55                  60                  65                  70
```

```
tgg ttt aca gag aat ggt gca aat aaa atc gga aag ctc tca aaa aaa   296
Trp Phe Thr Glu Asn Gly Ala Asn Lys Ile Gly Lys Leu Ser Lys Lys
                75                  80                  85

ggt ggc ttt aca gaa tat cca ttg cca cag ccg gat tct ggt cct tac   344
Gly Gly Phe Thr Glu Tyr Pro Leu Pro Gln Pro Asp Ser Gly Pro Tyr
            90                  95                 100

gga ata acg gaa ggt cta aat ggc gat ata tgg ttt acc caa ttg aat   392
Gly Ile Thr Glu Gly Leu Asn Gly Asp Ile Trp Phe Thr Gln Leu Asn
        105                 110                 115

gga gat cgt ata gga aag ttg aca gct gat ggg act att tat gaa tat   440
Gly Asp Arg Ile Gly Lys Leu Thr Ala Asp Gly Thr Ile Tyr Glu Tyr
    120                 125                 130

gat ttg cca aat aag gga tct tat cct gct ttt att act tta ggt tcg   488
Asp Leu Pro Asn Lys Gly Ser Tyr Pro Ala Phe Ile Thr Leu Gly Ser
135                 140                 145                 150

gat aac gca ctt tgg ttc acg gag aac caa aat aat tct att gga agg   536
Asp Asn Ala Leu Trp Phe Thr Glu Asn Gln Asn Asn Ser Ile Gly Arg
                155                 160                 165

att aca aat aca ggg aaa tta gaa gaa tat cct cta cca aca aat gca   584
Ile Thr Asn Thr Gly Lys Leu Glu Glu Tyr Pro Leu Pro Thr Asn Ala
            170                 175                 180

gcg gct cca gtg ggt atc act agt ggt aac gat ggt gca ctc tgg ttt   632
Ala Ala Pro Val Gly Ile Thr Ser Gly Asn Asp Gly Ala Leu Trp Phe
        185                 190                 195

gtc gaa att atg ggc aac aaa ata ggt cga atc act aca act ggt gag   680
Val Glu Ile Met Gly Asn Lys Ile Gly Arg Ile Thr Thr Thr Gly Glu
    200                 205                 210

att agc gaa tat gat att cca act cca aac gca cgt cca cac gct ata   728
Ile Ser Glu Tyr Asp Ile Pro Thr Pro Asn Ala Arg Pro His Ala Ile
215                 220                 225                 230

acc gcg ggg aaa aat agc gaa ata tgg ttt act gaa tgg ggg gca aat   776
Thr Ala Gly Lys Asn Ser Glu Ile Trp Phe Thr Glu Trp Gly Ala Asn
                235                 240                 245

caa atc ggc aga att aca aac gac aaa aca att caa gaa tat caa ctt   824
Gln Ile Gly Arg Ile Thr Asn Asp Lys Thr Ile Gln Glu Tyr Gln Leu
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caa aca gaa aat gcg gaa cct cat ggt att acc ttt gga aaa gat gga   872
Gln Thr Glu Asn Ala Glu Pro His Gly Ile Thr Phe Gly Lys Asp Gly
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tcc gta tgg ttt gca tta aaa tgt aaa att ggg aag ctg aat ttg aac   920
Ser Val Trp Phe Ala Leu Lys Cys Lys Ile Gly Lys Leu Asn Leu Asn
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gaa tga gatgggagtg agcaatattt atg aaa tgg caa aat cag caa ggc ccc 973
Glu                         Met Lys Trp Gln Asn Gln Gln Gly Pro
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aat cca gaa gaa ata tac cct ata gaa ggt aat aaa cat gtt caa ttt   1021
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Asn Pro Glu Glu Ile Tyr Pro Ile Glu Gly Asn Lys His Val Gln Phe
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Ile Lys Pro Ser Ile Thr Lys Pro Asn Ile Leu Val Gly Glu Tyr Ser
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tat tac gat agt aaa gat ggt gaa tct ttt gaa agc caa gtt ctt tat   1117
Tyr Tyr Asp Ser Lys Asp Gly Glu Ser Phe Glu Ser Gln Val Leu Tyr
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His Tyr Glu Leu Ile Gly Asp Lys Leu Ile Leu Gly Lys Phe Cys Ser
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att gga ccc gga acg aca ttt ata atg aat ggg gct aat cat cgt atg   1213
Ile Gly Pro Gly Thr Thr Phe Ile Met Asn Gly Ala Asn His Arg Met
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gat ggt tca aca ttt cca ttc aat ctt ttc gga aat ggt tgg gag aag   1261
Asp Gly Ser Thr Phe Pro Phe Asn Leu Phe Gly Asn Gly Trp Glu Lys
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cat acc cct aca ttg gaa gac ctt cct tat aag ggt aac acg gaa att   1309
His Thr Pro Thr Leu Glu Asp Leu Pro Tyr Lys Gly Asn Thr Glu Ile
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ggg aac gat gtt tgg att gga cga gat gtg aca att atg ccc ggt gta   1357
Gly Asn Asp Val Trp Ile Gly Arg Asp Val Thr Ile Met Pro Gly Val
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Lys Ile Gly Asn Gly Ala Ile Ile Ala Ala Lys Ser Val Val Thr Lys
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aag ata agg ttt tcc aag gaa aaa atc gca gca tta cta aaa gta agg   1501
Lys Ile Arg Phe Ser Lys Glu Lys Ile Ala Ala Leu Leu Lys Val Arg
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tgg tgg gac cta gag ata gag acg ata aat gaa aat att gat tgc atc   1549
Trp Trp Asp Leu Glu Ile Glu Thr Ile Asn Glu Asn Ile Asp Cys Ile
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